BEHAVIOR OF RATS OF DIFFERENT AGES ON A VITAMIN G DEFICIENT DIET

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FOUR FIGURES

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Although recognized for several years as an essential factor in good nutrition, vitamin G still presents many unknown phases. During the 16 years since Goldberger ('16) conducted his classical experiments which showed that pellagra in man is of dietary origin, little has been accomplished toward a completely satisfactory explanation of this disease. Attempts to produce pellagra experimentally in laboratory animals or to prove conclusively that vitamin G deficiency in rats is analogous to pellagra in humans have always furnished insufficient evidence to absolutely establish such correlations.

The histological work of Denton ('28) has led to the general acceptance that black-tongue in dogs and pellagra are of similar origin and nature. However, Gurin and Eddy ('31) report that the histological picture of rats suffering from vitamin G deficiency is different from the one seen in cases of human pellagra. On the other hand, Findlay ('28) describes 'pellagra-like' lesions occurring in rats deprived of vitamin G. Guha ('31) in discussing the etiology of pellagra points to the lack of clarity in evidence necessary for the acceptance of pellagra as the result of an uncomplicated deficiency of vitamin B_2 (G). Not infrequently the condition produced in rats on vitamin G deficient diets is referred to as pellagra. It is true that foods testing high in vitamin G

are usually found classified as good pellagra-preventive substances; and many of the symptoms, in so far as they have been compared, appear to be quite similar in the two deficiencies.

Goldberger and co-workers ('20) found an age as well as sexual and seasonal variation during a study of pellagrous patients in South Carolina. They noted that the disease was rare in children of 2 years or younger, and more prevalent in children between the ages of 2 and 10 years than in persons of 10 to 19 years, inclusive. Up to 20 years the incidence in both males and females was similar while between the ages of 20 and 54 years pellagra occurred many times more frequently in females than in males. There was a seasonal onset of the disease confined almost exclusively to the period from April to September wi'h a well-defined peak in June.

Thatcher and co-workers ('31) report a seasonal variation in their rats on vitamin G deficient diets while Leader ('30) was unable to produce symptoms of vitamin G deficiency during December, January, and February. In reviewing the literature, no reference has been found to work which had as its purpose a study of the effect of insufficient amounts of vitamin G upon rats of different ages and sex.

EXPERIMENTAL

The experiments reported in these studies were carried out with the idea of obtaining some additional knowledge concerning the behavior of rats of different ages and sex on a diet deficient in vitamin G. Animals of 4, 6, 8, 10, and 12 weeks and 6, 9, 12, 15, and 18 months old were selected for study. Only a representative number of rats from a given litter were placed on the experimental diet at 4 weeks of age; the remaining were kept on the stock ration until they were 6 weeks old when a similar number were removed and so on until the litter was as completely distributed between the various age groups as was possible. In the case of the older animals it was impossible to adhere strictly to this method

of sampling. However, in every case the animals selected for study in any one group were representative of several litters. Table 1 gives the number of animals, distribution of sex and average weight of the members of each age group at the beginning of the test.

The basal diet used in this study was that described by Sherman and Spohn ('23) and had the following composition: casein, 18 per cent; cod-liver oil, 2 per cent; butter-fat, 8 per cent; Osborne and Mendel salt mixture, 4 per cent; and cornstarch, 68 per cent. This diet was supplemented with 0.25 gm. of rice polish which according to Munsell ('29)

TABLE 1

Number, age, average initial weight and sex distribution of the rats used

AGE OF ANIMALS	NUMBER OF ANIMALS	DISTRIBUTION OF SEX		AVERAGE INITIAL WEIGHT
				Gm.
4 weeks	8	4 M	4 F	57.3
6 weeks	8	4 M	4 F	85.0
8 weeks	8	4 M	4 F	131.5
10 weeks	8	4 M	4 F	164.6
12 weeks	8	4 M	4 F	180.9
6 months	8	4 M	4 F	290.8
9 months	4	2 M	2 F	290.8
12 months	6	3 M	3 F	317.2
15 months	8	4 M	4 F	288.5
18 months	8	4 M	4 F	326.6

furnishes a fair amount of vitamin B and a very small amount of vitamin G, thereby making vitamin G the first limiting factor. Consequently this means that all of the animals regardless of their initial weight or age received the same amount of vitamin G.

The criteria used for comparing the behavior of animals of different ages on this diet low in vitamin G were the ability of the younger rats to grow and of the older ones to maintain weight as well as the survival period of each group. At death, gross autopsy findings were noted.

The average change in weight for each age group over the entire survival period is shown in figure 1. Normal animals

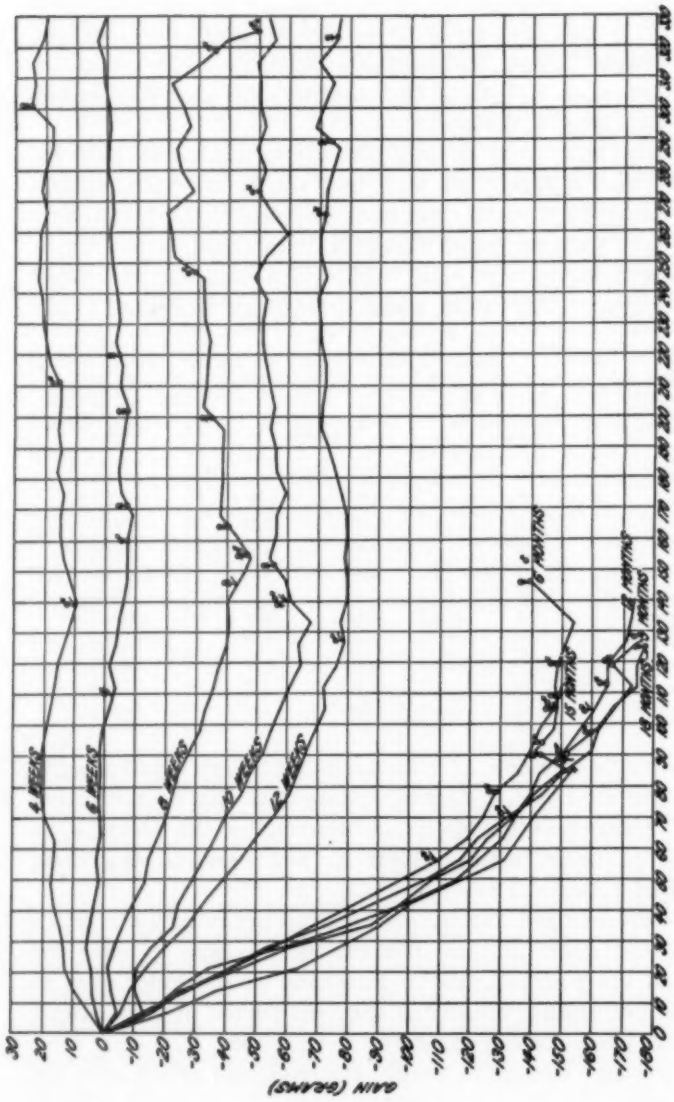

Fig. 1 Curves representing growth responses of groups of rats of various ages placed on a diet deficient in vitamin G. The ages of the animals at the beginning of the experiment are shown on the respective curves. The checks indicate the time of occurrence of the first death in the group, while the circles mark the deaths of the remaining animals.

4 weeks old when placed on this diet, slowly gained weight (about 20 gm. in all) during the first 3 months and thereafter throughout the remainder of the year maintained approximately this weight. Animals 6 weeks old maintained their initial weight with very little variation for more than a year. The rats in the other eight age groups lost weight from the start. Up to 6 months of age, the older the animals the more rapid was the decline in weight. Groups between 6 and 18 months of age showed very little difference in their growth responses on the vitamin G deficient diet. In no case was there a continued decline in weight throughout the experiment. Always, after reaching a certain low weight which was characteristic for each age group the animals continued for many months with relatively little variation in weight.

Since there was a considerable difference between the average weights of the animals of the different age groups at the time when each was placed on the experimental diet, it was questionable whether a fair comparison could be made between the actual changes in weight by the different groups. An animal weighing over 300 gm. could much better afford to lose 20 gm. than one weighing 75 gm. Consequently it seemed desirable to study and compare weight changes in terms of percentage of the initial weights of the animals. Calculations were made on the basis of average gains and not for each individual gain with subsequent averaging. These comparisons are shown in figure 2.

In every case up to 6 months of age, the older the rat the greater was the percentage loss of initial weight. The relationship between the age of the animals and their percentage weight loss was not a linear function, however. There were considerably greater differences in percentage losses between the groups of animals 4, 6, and 8 weeks old than between those 8, 10, and 12 weeks of age. In fact these latter groups showed very comparable percentage losses in weight.

Account was also taken of the fact that at the beginning of the experiment each of the younger rats possessed an innate impetus to grow, the amount of course depending upon

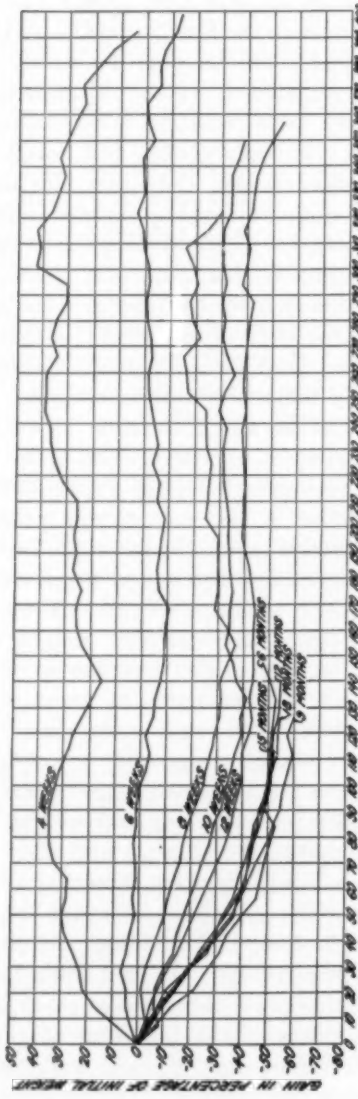

Fig. 2 Curves representing growth responses expressed as percentages of the initial weight of rats of different ages on a diet deficient in vitamin G. The age of each group of animals at the beginning of the experiment is shown along the curve for that group.

the age of the animal. In order to compare the behavior of the different age groups, the growth response of each was compared with that of normal rats of the same age and sex. The extent to which animals fell short of what could have been expected of them under normal conditions was then noted. Expressing normal growth by a base line, curves were plotted showing the average amount each group failed of reaching its normal weight. Such comparisons for male rats are shown in figure 3. The most outstanding point observed is that vitamin G insufficiency causes a great divergence from the normal growth of animals of all ages between 4 weeks and 18 months. By the end of the twelfth week, rats of the age groups under consideration showed an average weight that was between 157 and 218 gm. less than the weight normally expected.

Data relative to the length of survival show that older animals were unable to live for as long periods as those 3 months of age or younger. All of the rats died long before their normal life span had been covered. These data are incorporated in figure 1. It will be noticed that the survival behavior of all groups up to and including those 3 months of age were very similar. Twenty weeks had elapsed before at least one death had occurred in each of the younger groups of rats. The earliest death was during the sixteenth week in the group started at an age of 6 weeks. On the other hand, among the older animals the group of rats 15 months old at the beginning of the experiment suffered its first loss during the eighth week and all of these older groups had lost one animal by the twelfth week. The majority of the older animals were dead by the twentieth week.

From gross findings at autopsy it was concluded that the animals of all ages showed practically the same symptoms of vitamin G deficiency. Since all of the animals were subsisting on a diet which contained appreciable amounts of vitamin G, they did not develop as severe symptoms as rats which are given a diet totally devoid of this vitamin. In this respect the findings were more comparable with those occurring in

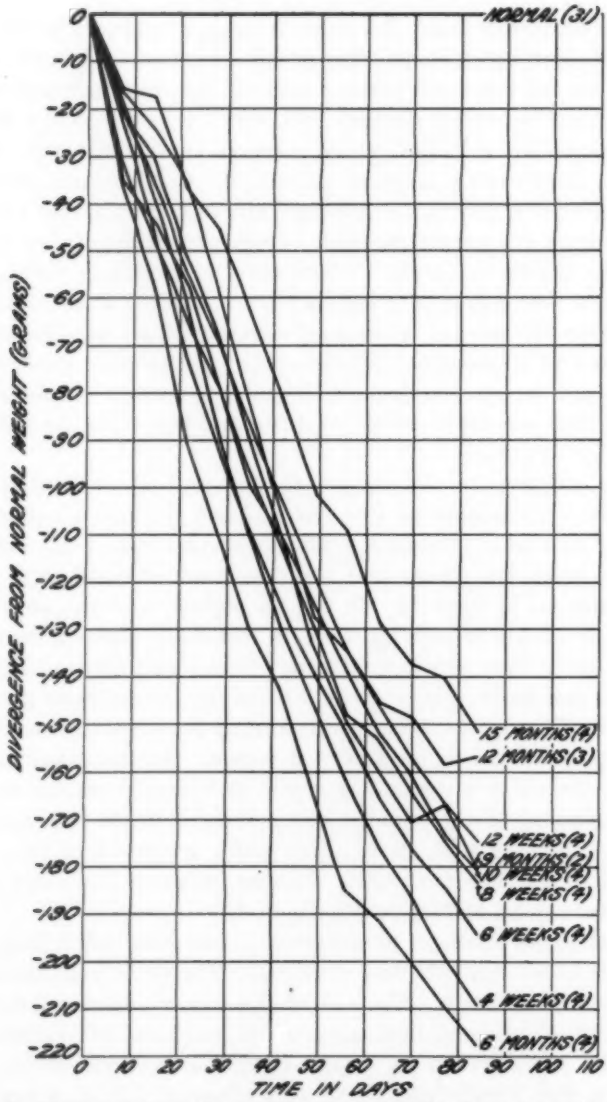

Fig. 3 Divergence from the normal gain in weight shown by male rats of different ages on a diet deficient in vitamin G. The age of the animals when put on the vitamin G deficient diet and the number in each group are indicated at the end of the curves.

cases of human dietary deficiencies where a vitamin is never entirely missing from the diet.

The usual development of the vitamin G deficient symptoms in the rats began with a loss in weight followed soon by a loss of hair and dermatitis. Lesions of various sizes appeared in a number of cases. These ocurred more frequently about the head, shoulders and legs of the animal but might be found on any part of the body. They were not bilaterally symmetrical however. There was incontinence of the urine which was highly pigmented and in the later stages often bloody in appearance. Priapism occurred in almost every case. Upon autopsy a pathological condition of the gastro-intestinal tract and liver was a marked characteristic of the disease. Generally, enormous hair balls were found in the stomach, and the walls of the cardiac portion were greatly thickened. Atrophy of the testes was usually observed. Except for a more pronounced tendency toward alopecia in the younger animals, there appeared to be no outstanding differences in the severity of the symptoms between the different age groups.

Comparing the responses of the males and females to vitamin G deficiency, among the younger animals, 4 and 6 weeks old, there was a tendency for the males to make slightly more rapid gains at first followed by a greater decline than that shown by the females. Both males and females of the 8-week-old group averaged almost an identical weight loss. In all of the older groups of animals 10 and 12 weeks and 6 to 18 months, the males suffered an increasingly greater actual loss in weight than the females (fig. 4). However, the fact must not be overlooked that male rats, especially the older ones, weighed much more at the beginning of the test than the females in the corresponding age groups. A comparison of weight changes based on the initial weight of the animals offers quite a different picture. Such a summary is given in table 2. Both males and females of the same age lost approximately the same percentage of their initial weight over a period of 16 weeks. This does not mean that animals in

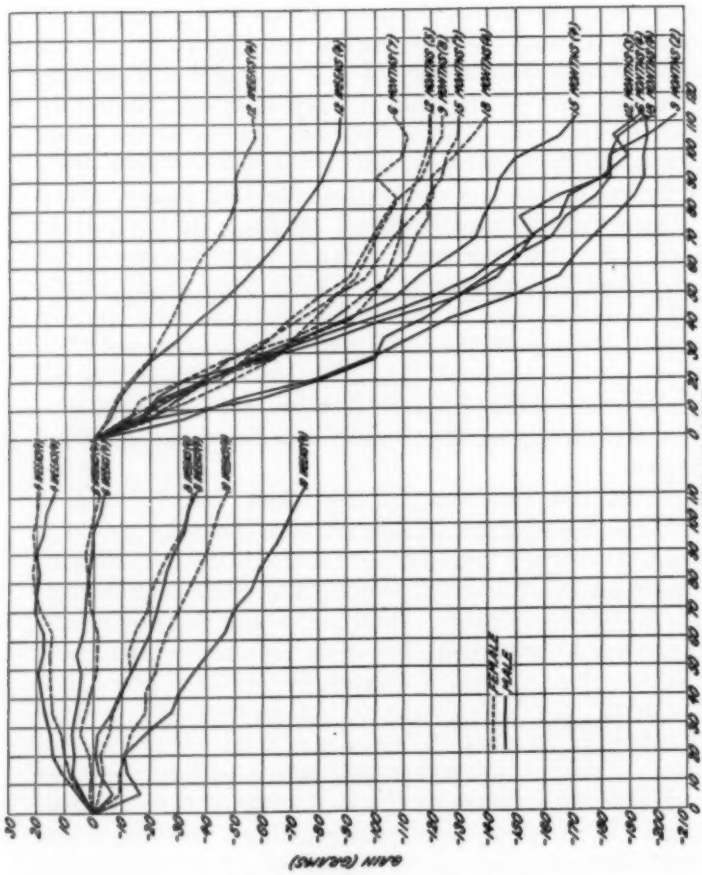

Fig. 4 Weight changes made by male and female rats of different ages on a diet deficient in vitamin G. The number of animals in the group and their age at the beginning of the test are indicated at the end of each respective growth curve.

TABLE 2

Changes in weight expressed as percentage of the initial weight of male and female rats between 4 weeks and 18 months of age on a diet deficient in vitamin G

AGE AT BEGINNING OF TEST	SEX	WEEKS ON EXPERIMENTAL DIET															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
4 weeks	F	8.1	15.4	18.1	19.9	25.7	27.1	27.1	27.5	26.6	32.5	36.5	38.3	36.5	38.0	35.6	34.4
	M	8.8	18.5	24.5	25.3	28.7	30.4	32.9	29.5	29.5	34.6	33.7	32.0	34.1	29.8	27.8	22.8
6 weeks	F	1.2	0.6	1.9	5.3	3.7	1.2	− 1.5	− 1.9	− 1.9	0.4	1.9	1.6	3.1	1.2	− 0.9	− 3.1
	M	4.2	8.2	7.0	8.2	6.7	5.6	4.8	6.5	4.2	3.1	2.6	1.1	0.3	0	− 3.0	− 4.5
8 weeks	F	−1.7	− 2.8	− 2.1	− 4.6	− 5.7	− 8.3	−11.1	−10.3	−12.4	−15.7	−17.0	−19.6	−22.8	−26.1	−27.8	−29.5
	M	−4.7	− 1.6	− 0.6	− 1.0	− 4.5	− 6.8	− 9.3	−11.9	−14.0	−15.7	−17.0	−18.1	−20.1	−22.5	−23.2	−25.7
10 weeks	F	−7.5	− 7.3	− 8.9	−10.7	−14.7	−14.7	−17.5	−18.7	−20.4	−23.8	−26.3	−28.7	−32.1	−33.5	−35.5	−37.5
	M	−8.3	− 5.9	− 5.4	− 9.1	−13.7	−15.0	−17.6	−20.6	−23.1	−24.3	−27.4	−28.8	−31.4	−33.2	−34.8	−36.5
12 weeks	F	−3.4	− 5.8	− 8.9	−13.6	−16.6	−19.0	−21.0	−23.7	−25.9	−30.0	−33.0	−34.6	−34.4	−36.4	−39.0	−38.3
	M	−2.7	− 4.2	− 6.9	− 9.6	−14.4	−17.6	−21.6	−25.2	−28.4	−31.2	−33.5	−35.7	−38.0	−39.6	−40.6	−41.0
6 months	F	−5.1	−11.9	−20.7	−24.7	−29.3	−32.2	−36.4	−41.8	−43.4	−45.3	−47.7	−49.6	−46.0	−50.2	−51.5	−49.3
	M	−8.9	−14.2	−22.1	−27.3	−31.7	−35.6	−41.2	−46.2	−47.6	−49.5	−51.6	−53.5	−54.4	−54.4	−54.8	−54.7
9 months	F	−8.9	−13.0	−16.7	−21.0	−27.2	−31.8	−36.6	−40.4	−42.0	−43.5	−45.0	−46.7	−50.3	−51.4	−53.3	−53.5
	M	−5.1	− 6.9	−11.8	−16.7	−22.8	−28.9	−34.5	−39.0	−41.8	−45.2	−43.7	−47.5	−52.7	−53.0	−56.7	−59.6
12 months	F	−8.0	−12.6	−16.3	−25.8	−31.5	−37.7	−40.2	−42.5	−43.6	−44.5	−44.8	−46.2	−47.1	−48.2	−48.8	−48.9
	M	−4.4	− 5.8	− 8.8	−15.7	−22.3	−28.1	−33.8	−36.2	−39.3	−40.5	−43.2	−43.9	−47.2	−49.4	−48.0	−50.5
15 months	F	−7.1	−14.0	−19.5	−24.9	−28.1	−33.4	−37.2	−41.2	−43.7	−44.9	−46.7	−46.6	−48.4	−49.0	−50.8	−50.8
	M	−4.4	− 8.3	−12.6	−17.0	−22.6	−27.5	−32.6	−34.9	−38.5	−41.6	−42.3	−43.5	−44.1	−46.1	−50.5	−52.3
18 months	F	−2.8	− 6.2	−12.0	−17.5	−26.4	−29.3	−31.3	−35.9	−37.8	−39.4	−42.1	−44.9	−44.6	−47.6	−49.9	−51.9
	M	−4.9	−15.4	−21.4	−26.0	−26.9	−30.6	−33.5	−37.3	−38.9	−42.2	−43.7	−46.3	−47.7	−47.7	−48.5	−51.1

different age groups made the same response since it has been shown earlier in this paper that the older the animal up to 6 months, the greater was the percentage weight loss suffered.

The data in figure 1 show no evidence of a difference in the survival of the two sexes on a diet low in vitamin G.

Contrary to the findings of Leader ('30) and Thatcher ('31) our animals showed no differences in behavior with the seasons. An explanation for the seasonal variation in the incidence of pellagra in human beings noted by Goldberger ('20) may be readily explained by the change in diet of these people during the year. Following the months in which fresh fruits and vegetables are less plentiful, severe outbreaks of the disease occur. However, in the case of the experimental animals whose diet and living conditions are practically constant at all times, it does not seem plausible to expect a seasonal change in their behavior.

CONCLUSIONS

Groups of rats ranging in age from 1 to 18 months inclusive were placed on a diet deficient in vitamin G. Data show that:

1. Animals 4 weeks old at the beginning of the test gained about 20 gm. during the first 3 months and thereafter maintained approximately this weight for the remainder of the year. Rats 6 weeks old maintained their initial weight for more than a year with little variation. Rats 8, 10, and 12 weeks, and 6, 9, 12, 15, and 18 months lost weight from the start. The older the animal up to 6 months of age, the more rapid was the loss. Age groups between 6 and 18 months showed very similar weight losses. After reaching a certain low weight level characteristic for each group, the animals maintained an approximately constant weight throughout the test.

2. Up to 6 months of age the older the animal the greater was the percentage of its initial weight lost. Greater differences in percentage losses occurred between animals 4, 6, and 8 weeks old than between those 8, 10, and 12 weeks of age.

The older groups showed the same percentage losses in weight.

3. By the end of the twelfth week of the test, the male rats in the various age groups were 157 to 218 gm. below the weight of normal animals of the same age and sex.

4. Groups of rats with initial ages of 4 to 12 weeks suffered only one loss from each group by the twentieth week while most of the animals in groups 6 to 18 months were dead. While the younger animals survived longer than the older ones, both died long before their normal life span had been covered.

5. Except for a tendency to more marked alopecia in the younger animals, the various age groups showed no difference upon autopsy in the type or severity of the gross symptoms of vitamin G deficiency.

6. Male and female rats of the same age, between 4 weeks and 18 months, made very comparable responses when these are considered as percentage of the initial weight gained or lost. The male rats, possessing greater initial weights, showed greater actual weight losses. In the younger animals there was a tendency for the males to make slightly greater responses than the females followed by a more rapid decline. The survival behavior of the two sexes appears to be the same for a given age.

7. There was no seasonal variation noted in the development of symptoms of vitamin G deficiency.

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STUDIES IN HUMAN PHYSIOLOGY

V. URINE CHEMISTRY; COMPARISON OF 24-HOUR AND SHORT-PERIOD, BASAL EXCRETION; CORRELATIONS RETWEEN URINE CONSTITUENTS AND MENSTRUAL AND SEASONAL VARIATION

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TWO FIGURES

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In the preceding reports of this series (Griffith et al., '29) will be found a record of the intra-individual variations of body temperature and basal metabolism, pulse rate and blood pressure, alveolar air, blood gas capacity and respiration of five normal adults who were under observation during the 2-year period, February, 1925, through January, 1927. Two of the subjects, A.B. and C.D., were men and served continuously throughout the entire period; the remaining three subjects were women and of them, two, E.F. and G.H., were under observation during the first year (February, 1925, through January, 1926), while the third, K.L., took their place during the second year (February, 1926, through January, 1927). For the first year, which will hereafter be referred to as '1925' (February, 1925, through January, 1926) there are, therefore, contemporaneous data for two men (A.B. and C.D.) and two women (E.F. and G.H.); during the succeeding year, '1926' (February, 1926, through January, 1927) the group was composed of the same two men and the third woman, K.L.; the first paper of this series should be consulted for further details in regard to the age, weight, and occupation.

It is the purpose of this report to present a summary of the data derived from analysis of the urine of these subjects.

METHOD

The primary purpose of this study was to determine the extent of variability that might be expected under normal conditions. To that end no observations are reported here that were made when there was any doubt as to the physical fitness of the subjects. Nor were there any restrictions as to diet, except one; viz., no meat was eaten during the day of the collection of the 24-hour urines during the first year, nor, as will be explained later, during the day preceding the collection of the short-period, morning urines of the second year; otherwise the subjects ate according to their desire both as to amount and kind of food. This single restriction was imposed in order to eliminate the possibility of an unusual stimulation of the basal metabolic rate, which was one of the chief matters under investigation, through the specific dynamic action of an occasional, excessive protein ingestion. The results would seem to indicate that protein intake was not unduly lowered, being kept normal, but, it is hoped, never becoming excessive, through the meat substitutes (eggs, milk, etc.) employed.

The data for 1925 are derived from 24-hour urines that were collected approximately once a week throughout the year during a day of no meat ingestion, as mentioned above.

Following this day during which no meat was eaten, the subjects came to the laboratory, the first thing in the morning, for determination of the basal metabolism and other measurements as previously reported. During 1926, these determinations were made approximately twice a week on each of the three subjects then serving. And during this year, instead of 24-hour urines, samples were obtained that corresponded with the period of the metabolism determinations; the bladder was emptied, noting the time, just before lying down; the urine was again passed and saved, and the time noted, upon getting up. The preliminary rest period preceding the

metabolism determinations was about 1 hour; the determination of the metabolic rate and the other measurements which were made in connection with it occupied approximately another hour (see the last item of table 1); so these (1926) samples represent the urine secretion under basal conditions early in the morning and after a day of controlled protein intake.

Since A.B. and C.D. served through both years without change in habit or diet, their data are particularly instructive for the comparison which they afford between 24-hour excretion and that occurring under basal conditions.

The methods of analysis were all standard and descriptions of them may be found in the following citations from Hawk and Bergeim's Practical Physiological Chemistry, ninth edition:

Urea: Aeration method of Cullen and Van Slyke, page 720.
Ammonia: Permutit method of Folin and Bell, page 727.
Creatinine: Folin's colorimetric method, page 733.
Creatin: Folin's method, page 735.
Uric acid: Colorimetric method of Benedict and Frank, page 736.
Amino acids: Colorimetric method of Folin, page 731.
Total nitrogen: Method of Folin and Wright, page 713.
Organic acids: (Corrected for creatinine and amino acids) Van Slyke and Palmer, page 706.
Total acid: Folin's method, page 705.
Sugar: Folin and Berglund, page 753.
Chlorides: Whitehorn, page 401.
Phosphorus: Fiske and Subbarow, page 776.

All analyses were done with standardized apparatus in duplicate and repeated when necessary until the customary checks were obtained.

I. STATISTICS

1. Averages; see table 1. In using the table it should be noticed that the data from the short-period urines of 1926 have been computed to a 24-hour basis in order to facilitate comparison with the 24-hour samples of 1925.

TABLE 1

Statistics of urinary excretion. The urines of 1925 were 24-hour, and those of 1926 short-period, morning, basal samples (the last entry of the table gives the average times of collection). For purposes of comparison all values are in terms of excretion per 24 hours

FUNCTION	SUBJECT	YEAR	NUMBER OF DETERMINATIONS	CUBIC CENTIMETERS AND GRAMS PER 24 HOURS			STANDARD DEVIATION	COEFFICIENT OF VARIATION
				Minimum and maximum	Mode	Arithmetical mean		
Volume cubic centimeters per 24 hours	A.B.	1925	52	550–1850	1000	1000±25	272.0	24.7
		1926	86	600–4800	1920; 2280; 2640 2880; 3240	2554±60	829.6	32.5
	C.D.	1925	51	650–2000	1050; 1150	1086±23	242.0	22.3
		1926	85	480–2400	960	1080±29	394.8	36.6
	E.F.	1925	48	400–2100	650	950±32	325.0	34.2
	G.H.	1925	46	350–1200	600	663±16	159.5	24.1
	K.L.	1926	81	240–5160	1920; 3000	2344±98	1308.0	56.0
Specific gravity	A.B.	1925	50	1.005–1.038	1.021	1.0219±0.0004	0.006	0.54
		1926	86	1.002–1.026	1.010; 1.011	1.0115±0.0003	0.004	0.43
	C.D.	1925	51	1.012–1.038	1.025	1.0243±0.0005	0.005	0.50
		1926	82	1.008–1.036	1.024	1.0116±0.0004	0.005	0.49
	E.F.	1925	48	1.011–1.031	1.018	1.0212±0.0006	0.006	0.57
	G.H.	1925	45	1.012–1.039	1.027	1.0259±0.0007	0.007	0.69
	K.L.	1926	78	1.002–1.039	1.008; 1.010	1.0135±0.0006	0.010	0.94
Urea N grams per 24 hours	A.B.	1925	52	5.60–11.00	7.4; 7.8	7.760±0.110	1.13	14.5
		1926	86	5.28–11.76	8.52	8.200±0.090	1.23	15.0
	C.D.	1925	49	5.20–13.60	8.80	8.580±0.150	1.52	17.8
		1926	85	5.52–12.24	8.40	7.870±0.090	1.19	16.1
	E.F.	1925	48	3.00– 7.20	5.00	5.442±0.094	0.97	17.8
	G.H.	1925	46	2.60– 9.00	5.60	5.448±0.144	1.45	26.6
	K.L.	1926	81	1.92–11.28	7.20	7.090±0.126	1.68	23.7
Ammonia-N gram per 24 hours	A.B.	1925	52	0.20–0.61	0.43	0.397±0.009	0.091	23.0
		1926	86	0.12–0.65	0.26	0.285±0.006	0.086	30.3
	C.D.	1925	51	0.40–0.84	0.48	0.560±0.010	0.105	18.7
		1926	85	0.10–0.96	0.38	0.469±0.013	0.177	37.7
	E.F.	1925	48	0.24–0.74	0.46	0.483±0.010	0.104	21.5
	G.H.	1925	44	0.12–0.84	0.24	0.282±0.015	0.144	51.1
	K.L.	1926	81	0.00–0.50	0.22	0.214±0.007	0.093	43.4
Creatinine-N grams per 24 hours	A.B.	1925	52	0.20–0.62	0.43; 0.44	0.442±0.005	0.053	12.7
		1926	83	0.31–0.50	0.41	0.415±0.002	0.033	8.1
	C.D.	1925	51	0.43–0.80	0.54; 0.56	0.580±0.007	0.075	12.9
		1926	85	0.41–0.73	0.61	0.600±0.004	0.051	8.4
	E.F.	1925	48	0.27–0.52	0.36; 0.38; 0.39 0.42	0.384±0.004	0.045	11.8
	G.H.	1925	46	0.15–0.44	0.29; 0.37; 0.44	0.323±0.008	0.078	24.2
	K.L.	1926	79	0.12–0.43	0.35	0.358±0.003	0.043	12.2
Creatine-N grams per 24 hours	A.B.	1925	51	0–0.092	0.022; 0.030	0.041±0.002	0.022	53.5
		1926	76	0–0.050	0	0.012±0.001	0.013	104.0
	C.D.	1925	51	0–0.120	0.035	0.049±0.003	0.029	60.0
		1926	80	0–0.086	0	0.027±0.002	0.023	86.2
	E.F.	1925	47	0.004–0.086	0.048	0.047±0.002	0.021	44.2
	G.H.	1925	45	0–0.180	0.050	0.045±0.004	0.035	78.0
	K.L.	1926	75	0–0.089	0	0.022±0.002	0.019	86.8
Uric acid-N grams per 24 hours	A.B.	1925	52	0.070–0.260	0.150	0.162±0.004	0.043	26.3
		1926	84	0.084–0.372	0.180	0.188±0.003	0.037	19.8
	C.D.	1925	51	0.110–0.330	0.150	0.185±0.004	0.047	25.6
		1926	85	0.108–0.528	0.228	0.220±0.004	0.054	24.6
	E.F.	1925	48	0.080–0.250	0.160	0.158±0.004	0.039	24.7
	G.H.	1925	46	0.030–0.240	0.120	0.127±0.004	0.045	35.6
	K.L.	1926	78	0.036–0.204	0.156	0.153±0.003	0.033	21.6

TABLE 1—*Continued*

FUNCTION	SUBJECT	YEAR	NUMBER OF DETERMINATIONS	CUBIC CENTIMETERS AND GRAMS PER 24 HOURS			STANDARD DEVIATION	COEFFICIENT OF VARIATION
				Minimum and maximum	Mode	Arithmetical mean		
Amino acid-N grams per 24 hours	A.B.	1925	51	0.050–0.164	0.082	0.098±0.002	0.023	23.7
		1926	82	0–0.149	0.082; 0.094 0.108; 0.132	0.098±0.002	0.027	27.5
	C.D.	1925	51	0.085–0.185	0.100	0.120±0.002	0.025	20.9
		1926	85	0.066–0.174	0.108	0.110±0.002	0.030	27.1
	E.F.	1925	48	0.060–0.290	0.100	0.114±0.004	0.042	37.1
	G.H.	1925	46	0.040–0.200	0.060; 0.080	0.090±0.004	0.036	39.4
	K.L.	1926	75	0.036–0.156	0.096	0.098±0.000	0.021	21.5
Total nitrogen grams per 24 hours	A.B.	1925	51	7.0–12.6	9.6; 10.0	9.40±0.11	1.17	12.5
		1926	84	7.0–13.0	8.8; 9.2; 9.5; 9.8	9.30±0.09	1.21	13.0
	C.D.	1925	51	7.4–16.4	11.6	10.63±0.16	1.73	16.3
		1926	85	6.7–15.1	10.3	9.83±0.10	1.39	14.1
	E.F.	1925	48	4.8– 9.2	6.6	7.09±0.11	1.11	15.6
	G.H.	1925	46	3.2–10.4	6.6	6.85±0.17	1.68	24.7
	K.L.	1926	81	2.4–12.7	9.6	8.35±0.13	1.74	20.8
Rest nitrogen grams per 24 hours	A.B.	1925	51	0.050–1.350	0.350	0.462±0.025	0.268	58.0
		1926	75	0.024–0.984	0.288; 0.300	0.450±0.022	0.286	63.6
	C.D.	1925	49	0.200–1.150	0.550	0.600±0.020	0.212	35.3
		1926	84	0.072–1.512	0.624	0.583±0.018	0.239	41.0
	E.F.	1925	47	0.100–1.500	0.350	0.472±0.027	0.270	57.2
	G.H.	1925	45	0.050–1.300	0.300	0.449±0.029	0.285	63.5
	K.L.	1926	70	0.048–0.840	0.552	0.393±0.016	0.194	49.4
Organic acids cubic centimeters 0.1 N acid per 24 hours. Corrected for creatinine and amino acids	A.B.	1925	51	330–670	460	505.7± 7.8	82.5	16.3
		1926	73	0–756	420	468.8±12.5	158.4	33.8
	C.D.	1925	47	390–930	540; 570; 600; 610; 660	614.5±11.1	113.0	18.4
		1926	71	0–792	528	528.0±10.9	136.3	25.8
	E.F.	1925	45	350–930	550	569.3±11.5	115.0	20.2
	G.H.	1925	42	180–920	520	432.4±15.5	149.0	34.5
	K.L.	1926	62	276–744	408	493.5± 9.8	114.9	23.3
Sugar grams per 24 hours	A.B.	1925	52	0.280–1.000	0.520; 0.620	0.552±0.012	0.128	23.2
		1926	84	0.252–0.516	0.312; 0.336	0.361±0.004	0.058	16.0
	C.D.	1925	51	0.300–0.900	0.620	0.718±0.011	0.118	16.4
		1926	85	0.192–0.264	0.384	0.404±0.005	0.063	15.6
	E.F.	1925	47	0.200–0.820	0.560	0.479±0.012	0.120	25.1
	G.H.	1925	46	0.200–1.500	0.500	0.531±0.023	0.226	42.6
	K.L.	1926	78	0.108–0.528	0.396	0.352±0.006	0.075	21.4
Phosphate grams per 24 hours	A.B.	1926	84	0.156–0.756	0.372	0.371±0.008	0.113	30.5
	C.D.	1926	85	0.216–1.056	0.648	0.588±0.012	0.117	28.4
	K.L.	1926	76	0.216–1.200	0.552; 0.576	0.596±0.014	0.174	29.2
Chloride grams per 24 hours	A.B.	1926	82	5.4–24.6	12.0	15.024±0.337	4.52	30.1
	C.D.	1926	83	3.6–19.8	8.4; 9.6	9.558±0.244	3.30	34.5
	K.L.	1926	76	3.6–21.0	10.2	9.710±0.272	3.52	36.3
Total acid cubic centimeters 0.1 N acid per 24 hours	A.B.	1926	72	Alk.–288	36	70.0±4.4	55.7	79.5
	C.D.	1926	76	Alk.–456	84	163.6±4.0	51.1	31.2
	K.L.	1926	72	Alk.–504	84	153.6±8.3	103.9	19.3
Time of collection, 1926 Minutes	A.B.		86	90–186	134	135		
	C.D.		85	92–238	164	165		
	K.L.		81	94–180	120	134		

Aside from establishing norms for the intra-individual variation in excretion to be expected over a period of time, perhaps one of the most interesting uses of the table is the comparison which it affords between the rates of excretion under basal conditions in the morning (1926) and the average 24-hour rate (1925). This comparison can be strictly made only with the data of the two men, A.B. and C.D., since they only furnished both 24-hour and short-period urines; and, even with them, the comparison is subject to the limitation that the urines to be compared were secured a year apart. As mentioned before, however, it is believed that there were no differences in diet or habits during the 2 years sufficient to invalidate the comparison and that the averages for each year are based on a sufficient number of observations to exclude any gross errors due to limitation of sampling.

Assuming, then, that the data for A.B. and C.D. furnish an approximately valid comparison between the average rate of excretion during 24-hour periods and that under post-absorptive, basal conditions for short periods in the morning, it is somewhat surprising to find how little consistent difference they show. Ammonia, creatine and sugar are the only substances that have a definitely lowered rate of excretion in the basal morning urines. The maxima and minima, the modes and averages are either quite alike or fail to show consistent differences in the two subjects for the other data, with the exception of uric acid which shows a higher rate of excretion under basal conditions for both.

The volume of the urine under the two conditions is worthy of remark because of the exceptional behavior of C.D. With A.B., the rate of excretion of the short-period urines is, as an average, two and one-half times greater than the average 24-hour rate; this was to be expected both from the effect of recumbency (White, Rosen, Fischer and Wood, '26) and the fact that they were obtained in the morning (Simpson, '24). A comparison of the data for K.L. with that for the other two women subjects, E.F. and G.H., though by no means a very valid one, would seem, also, to substantiate the recumbent and matutinal effects on volume. With C.D., however, the

average 24-hour rate is almost exactly that computed from the short-period samples obtained during the morning while lying down.

This difference in the behavior of the volume may perhaps be of significance in explaining the findings in regard to urea, since, as is well known and will be more fully developed in a following section, the two are to a certain degree correlated. The rate of urea excretion of A.B. is less in the 24-hour samples than in those obtained under basal conditions in which the protein metabolism must have been minimal and predominantly endogenous; and a similar difference is again indicated, though less validly, by comparison of the rates of excretion of the three women subjects, E.F., G.H. (1925, 24-hour urines), and K.L. (1926, basal, morning urines). On the other hand, C.D., who fails to show the matutinal and recumbent augmentation of volume has a lower rate of excretion for urea under basal conditions (1926) than in the 24-hour samples (1925) where the amount is no doubt increased from exogenous sources.

The greater rate of creatine excretion in the 24-hour urines than under basal conditions may be taken as evidence in support of at least partly exogenous origin for the amount of this substance normally excreted (Hunter, '22, p. 610); and the same may be said for sugar (Folin and Berglund, '22 a). In addition, attention may be called to the almost identical average excretion of creatin by the men and women of this group; there is no evidence of a sex difference, except that the men are more variable, as will be shown in a following section.

The average 24-hour output of ammonia is no doubt raised by the high level of excretion during sleep which many have reported (Simpson, '24, '26); whereas the morning urines may have partaken of the alkaline tide following awakening (Hubbard, '30) with concomitant retention of this substance (Bazett, '24).

There is previous evidence to prepare us for the greater rate of uric acid excretion in the basal, morning urines than in the 24-hour samples; its cause is uncertain. It can hardly

be due to the matutinal diuresis, for, although it has been claimed (Goiffon, '28) that the concentration of uric acid tends to remain fixed, whatever the output of water, our own data do not substantiate this. In the first place the correlation between uric acid and volume, especially in the short-period urines, is too low to be significant (see below); and, secondly, the increased output in the morning urines is even more pronounced in the subject C.D. than with A.B., although the former showed no morning diuresis. Lennox ('25) has shown that the blood uric acid rises during a fast, but he was inclined to attribute this to retention, which would decrease rather than increase the urinary output; moreover, Hubbard ('27) has shown that within the time after the last meal represented by our morning urines (16 to 18 hours) this increase in blood concentration has not become noticeable. Finally, there is nothing except individual preference to guide one in choosing between the suggestion by Smetanka ('12, '13) of a delayed effect of the previous meal; and that of Cathcart, Kennaway and Leathes ('07) of increased muscular activity, if only of tonus, on awakening.

Creatinine and amino acids both show remarkably similar rates of excretion in the 24-hour and short-period, morning urines. This was not unexpected of the former and may be taken as confirmation of the views of Folin and of Shaffer (Hunter, '22, p. 593) regarding the constant metabolism of this substance. It may be mentioned as a matter of record that the creatinine coefficients of these subjects fall within expected ranges, being, for the men, 8.1 (A.B.) and 9.8 (C.D.) with an average of 8.9; and for the women, 7.2 (E.F.), 5.8 (G.H.), and 6.4 (K.L.), with an average of 6.5.

On the other hand so little is known in regard to amino acid metabolism that it is difficult to appraise the significance of the similar rates of excretion of this fraction in the 24-hour and short-period, morning urines. From the work of Folin and Berglund ('22 b), as well as other work to which they refer, it might have been expected that the 24-hour excretion would be augmented by the greater output occurring after meals, especially since there seems to be no threshold for these sub-

stances, and it is claimed for them (loc. cit., p. 411) that the rate of excretion is independent of diuresis. This last, however, may need revision and it is then not impossible that the conditions here are similar to those affecting urea as described above. This is suggested by the relatively high correlation (see below) between amino acids and urea and amino acids and volume (especially in the short-period samples) as well as the fact that C.D., who, as explained above, failed to show the matutinal diuresis, also has a slightly lower average rate of amino acid excretion in the morning urines than in the 24-hour samples.

2. Variability. The following tabulation affords a comparison, in terms of the coefficient of variation, of the variability of excretion of these substances in the 24-hour and short-period, basal morning urines. The figures are the averages for all subjects serving in each year, derived from table 1:

Coefficients of variation

1925 24-hour urines		*1926 Short-period urines*
Specific gravity	0.6	0.6 Specific gravity
		9.6 Creatinine
Creatinine	15.4	
		15.9 Total nitrogen
Total nitrogen	17.3	
		17.6 Sugar
		18.3 Urea
Urea	19.2	
		22.0 Uric acid
Organic acid	22.3	
		25.4 Amino acid
Volume	26.3	
Sugar	26.8	
		27.6 Organic acid
Uric acid	28.1	
Ammonia	28.6	
		29.4 Phosphates
Amino acids	29.8	
		33.6 Chlorides
		37.1 Ammonia
		41.7 Volume
		43.3 Total acid
		51.3 Rest nitrogen
Rest nitrogen	53.5	
Creatine	58.9	
		92.3 Creatine

These figures show that there is little significant difference either in the absolute or relative variability of the majority of these substances in the two types of urines. The only appreciable exceptions are the greater variability of sugar in the 24-hour urines and of volume (per unit time in both) in the short-period samples. It may be noted that creatine, the most variable constituent of both urines, is much more variable in the short-period, basal ones.

In the last of the previous numbers of this series (Griffith et al., '29 d, p. 569) may be found a table of the coefficients of variation of the physiological functions previously reported upon; these were twenty-nine in number and of them eighteen, or practically two-thirds, were more variable in the women than in the men. Attention may be recalled to the warning already given (Griffith et al., '29 c, p. 454; '29 d, p. 567) against uncritical generalization from such limited data as we have; nevertheless, merely as a description of our results, it is interesting to note that this apparent sex difference continues to hold in even greater proportion for the data presented here. This may be seen from the following tabulation compiled from the data of table 1:

Average coefficients of variation for the men and women of our group and grand averages for both sexes and both types of urine

	Men	*Women*	*Grand average*
[1] Specific gravity,	0.5	0.7	0.6
[1] Creatinine,	10.5	16.1	12.9
[1] Total nitrogen,	14.0	20.4	16.6
[1] Urea,	15.9	22.7	18.8
[1] Sugar,	17.8	29.7	22.9
[1] Organic acids,	23.6	26.0	24.6
[1] Uric acid,	24.1	27.3	25.5
[1] Amino acids,	24.8	32.7	28.0
Phosphates,	29.4	29.2	29.4
[1] Ammonia,	27.4	38.7	32.2
[1] Volume,	29.0	38.1	32.9
[1] Chlorides,	32.3	36.3	33.6
Total acid,	55.3	19.3	43.3
[1] Rest nitrogen,	49.5	55.7	52.6
Creatine,	75.9	67.3	73.2

[1] Women more variable than the men.

Thus, with the exception of phosphates, total acid and creatine, the average variability of the women is greater than that of the men. As was mentioned in the previous section, this greater variability of creatine excretion in the men than in the women is the only evidence these data afford in support of the widely held belief that creatinuria in the adult is essentially a female prerogative (see Hunter, '22, p. 612). As far as the actual, average amounts excreted over a period of time are concerned, the two sexes are quite alike, as may be seen from table 1.

Referring again to the table in the preceding report, it may be seen that for twenty-seven of the twenty-nine functions recorded there, the coefficients of variation range from 0.5 to 12.9. The remaining two, with coefficients greater than this (18.3), are for the protein oxygen and carbon dioxid, derivable from the data of this paper. It would appear, therefore, that the variability in excretion of the urinary constituents stands relatively high as compared with the metabolic, respiratory and cardio-vascular functions previously reported; for, of these urinary data, only the specific gravity and creatinine under basal conditions have coefficients of variation less than 12.9. That our coefficients are not abnormally high would seem to be apparent from a comparison with the only other data of similar kind, those of C. P. White, who gives the following figures, alongside which are placed our corresponding figures in parenthesis:

Urea,	30.7	(18.9)
Phosphates,	37.2	(29.4)
Chlorides,	44.3	(33.6)
Uric acid,	45.1	(25.5)
Ammonia,	50.0	(32.2)

3. Correlations. Following are given the coefficients of correlation for simultaneous variations in rate of excretion of the urinary constituents for which we have record. The left-hand entries are for 24-hour, and the right-hand for the short-period, basal, morning urines.

1. Correlations with volume

	+0.63	Volume, chlorides
	0.45	Volume, amino acids
	0.33	Volume, urea
Volume, creatinine	0.31	Volume, sugar
	0.29	Volume, creatine
Volume, urea	0.25	
Volume, uric acid	0.23	
Volume, amino acids	0.22	
Volume, organic acids	0.21	
	0.17	Volume, organic acids
	0.16	Volume, uric acid
	0.12	Volume, creatinine
Volume, creatine	0.08	
Volume, sugar	—0.01	
	0.02	Volume, ammonia
	0.08	Volume, phosphates
Volume, ammonia	0.13	

2. Correlations with urea

Urea, ammonia	+0.49	
Urea, uric acid	0.40	
	0.37	Urea, amino acid
Urea, creatinine	0.36	Urea, phosphate
	0.35	Urea, creatinine
	0.33	Urea, volume
	0.32	Urea, sugar
Urea, amino acids	0.31	
	0.30	Urea, uric acid
Urea, volume	0.25	Urea, chloride
	0.23	Urea, ammonia
		Urea, organic acid
Urea, organic acid	0.19	
	0.17	Urea, creatine
Urea, sugar	0.12	
Urea, creatine	0.01	

3. Correlations with ammonia

Ammonia, urea	+0.49	
	0.23	Ammonia, urea
	0.21	Ammonia, phosphate
Ammonia, sugar	0.16	
Ammonia, organic acids	0.15	
	0.14	Ammonia, creatinine
Ammonia, amino acids	0.10	
	0.07	Ammonia, sugar
Ammonia, creatinine	0.06	
	0.05	Ammonia, amino acids

Ammonia, creatine	0.02	Ammonia, creatine
Ammonia, uric acid		Ammonia, uric acid
	—0.02	Ammonia, chlorides
	0.11	Ammonia, volume
Ammonia, volume	0.13	Ammonia, organic acids

4. Correlataions with creatinine

Creatinine, uric acid	+0.54	
Creatinine, urea	0.36	
	0.35	Creatinine, urea
Creatinine, volume	0.31	
Creatinine, sugar		
Creatinine, organic acid	0.28	
	0.27	Creatinine, uric acid
	0.22	Creatinine, phosphates
	0.21	Creatinine, amino acids
Creatinine, amino acids	0.18	
	0.17	Creatinine, sugar
	0.14	Creatinine, ammonia
	0.12	Creatinine, volume
		Creatinine, organic acid
Creatinine, ammonia	0.06	
	—0.04	Creatinine, chloride
	0.11	Creatinine, creatine
Creatinine, creatine	0.23	

5. Correlations with creatine

	+0.29	Creatine, volume
Creatine, organic acid	0.28	
	0.24	Creatine, chloride
	0.22	Creatine, sugar
	0.17	Creatine, urea
Creatine, volume	0.08	
	0.06	Creatine, amino acid
	0.03	Creatine, phosphate
Creatine, ammonia	0.02	Creatine, ammonia
Creatine, urea	0.01	
Creatine, amino acid	—0.01	Creatine, uric acid
Creatine, uric acid	0.03	
	0.07	Creatine, organic acid
Creatine, sugar	0.11	Creatine, creatinine
Creatine, creatinine	0.23	

6. Correlataions with uric acid

Uric acid, creatinine	+0.54	
Uric acid, urea	0.40	
Uric acid, sugar	0.34	
	0.30	Uric acid, urea
	0.27	Uric acid, creatinine
	0.24	Uric acid, amino acid

Uric acid, volume	0.23	
Uric acid, organic acid	0.22	
Uric acid, amino acid	0.19	
	0.17	Uric acid, phosphates
	0.16	Uric acid, volume
		Uric acid, chlorides
	0.13	Uric acid, organic acids
	0.12	Uric acid, sugar
Uric acid, ammonia	0.02	Uric acid, ammonia
	—0.01	Uric acid, creatine
Uric acid, creatine	0.03	

7. Correlations with amino acids

	+0.45	Amino acids, volume
	0.37	Amino acids, urea
		Amino acids, sugar
Amino acids, organic acid	0.35	
	0.32	Amino acids, chlorides
Amino acids, urea	0.31	
	0.24	Amino acids, uric acid
Amino acids, volume	0.22	
	0.21	Amino acids, creatinine
	0.20	Amino acids, organic acid
Amino acids, uric acid	0.19	
Amino acids, creatinine	0.18	
Amino acids, ammonia	0.10	
	0.06	Amino acids, creatine
	0.05	Amino acids, ammonia
	0.04	Amino acids, phosphates
Amino acids, creatine	—0.01	
Amino acids, sugar		

8. Correlations with sugar

	+0.37	Sugar, amino acid
Sugar, uric acid	0.34	
	0.32	Sugar, urea
Sugar, creatinine	0.31	Sugar, volume
	0.23	Sugar, chlorides
Sugar, organic acid	0.22	Sugar, creatine
	0.17	Sugar, creatinine
Sugar, ammonia	0.16	
	0.14	Sugar, organic acid
Sugar, urea	0.12	Sugar, uric acid
	0.11	Sugar, phosphate
	0.07	Sugar, ammonia
Sugar, volume	—0.01	
Sugar, amino acid		
Sugar, creatine	0.11	

9. Correlations with chlorides

(Data for short-period, morning urines, only)

+0.63	Chlorides, volume
0.32	Chlorides, amino acid
0.25	Chlorides, urea
0.24	Chlorides, creatine
0.23	Chlorides, sugar
0.19	Chlorides, organic acid
0.16	Chlorides, uric-acid
0.02	Chlorides, ammonia
—0.03	Chlorides, phosphates
0.04	Chlorides, creatinine

10. Correlataions with phosphates

(Data for short-period, morning urines, only)

+0.36	Phosphates, urea
0.22	Phosphates, creatinine
0.21	Phosphates, ammonia
0.17	Phosphates, uric acid
0.11	Phosphates, sugar
0.09	Phosphates, organic acid
0.04	Phosphates, amino acid
0.03	Phosphates, creatine
—0.03	Phosphates, chlorides
0.08	Phosphates, volume

Too much is not to be made of the absolute size of these coefficients; those for the 24-hour urines are based on approximately 100 analyses and those for the short-period samples on about 225, so the probable errors range from ± 0.04 to $\pm$ 0.07, depending on the magnitude of the coefficient. The smaller coefficients are given, therefore, for comparative purposes only; in order, in the first place, to be able to compare the conditions that obtain in the 24-hour samples with such as are shown by the short-period ones; and, secondly, to compare our results with those that have been or may be reported by others.

Discussion. There is very little previous work directly bearing on this aspect of excretion. Rich has reported ('28) a correlation between creatinine and phosphorus, in 24-hour urines, of + 0.64. We do not have a strictly comparable figure, since ours of + 0.22 was obtained only on short-period

urines. Hubbard and Munford ('22) and Hubbard ('23, '24) have shown by a method of analysis somewhat different from ours, but still of a statistical nature, that ammonia excretion varies as the volume of the urine, provided the reaction is constant; otherwise, as in our work, there is no correlation. But the most comparable precedent is to be found in the exhaustive statistical analysis by C. P. White ('22, '25) of the data from 100 urines (presumably 24-hour), fifty of which were derived from hospital patients with cancer of the breast and fifty from hospital patients suffering from other diseases not affecting kidney function. The latter were used as controls in the effort to establish peculiarities of metabolism associated with cancer. For our purpose they may be taken to represent normal kidney function under hospital standardization of exercise and diet, and as such afford an interesting comparison with the data (24-hour) provided by our normal subjects under ordinary living conditions.

Coefficients of correlation

	24-hour urines	
	White Hospital patients 50 samples	Ourselves Normal subjects 100 samples
Volume, creatinine,	+0.54	+0.31
urea,	0.42	0.25
uric acid,	0.40	0.23
ammonia,	0.19	—0.13
Urea, creatinine,	+0.71	+0.36
uric acid,	0.41	0.40
ammonia,	0.38	0.49
Uric acid, creatinine,	+0.27	+0.54

Although all of our values in the first group are lower than White's, it is interesting that the same relative correlation with volume is evident in both. The absolute differences, here, as in the remaining correlations with urea and uric acid, may not improbably reflect the different physiological conditions represented by them.

It is not necessary or expedient to attempt a discussion of the very extensive literature which might bear in some way

on these results. Much of this can be found summarized in the review of Marshall ('26). Nevertheless, there are a few points that deserve attention.

It can be seen that the correlation between volume and chlorides is so much greater than any other as to be in a class apart, and what we have found for the short-period, morning urines (we have chloride determinations for them only) presumably obtains for 24-hour samples also; for C. P. White, in the work already referred to, gives the coefficient of correlation between volume and chlorides as +0.76. These are merely definite numerical statements of a fact known qualitatively long since. Thus, Adolph has stated ('23, p. 448) that as between urea and chloride, the latter is the chief factor in determining urine volume; and Griffith and Hansell ('25), Simpson ('24), Marshall and Crane ('22) and White, Rosen, Fischer and Wood ('26) have shown that in the diureses due to abdominal pressure, morning collection, section of splanchnic nerve and recumbency, chloride excretion is preeminently increased (the only exception is the diuresis due to ingestion of water or salt solution (Adolph, '21; H. L. White, '27) in which chloride excretion seems to be independent of volume, or, according to Marshall ('20), less augmented than the output of urea). But even where volume and chloride are related a correlation coefficient is of no value in determining causality. From this point of view, 24-hour urines probably present too complicated a tangle to be at present unraveled. Whether, under the simpler conditions represented by our basal, morning urines, water swept out chloride or the latter determined the volume, would seem to be decided in favor of the latter; for Simpson has shown ('26) that on waking from sleep in the morning the amount of chloride excreted markedly increases whether the volume does so or not; and this primary excess of chloride appears to arise from deep-seated physiological adjustments, particularly of respiration, that occur on transition from sleep to wakefulness (Simpson and Wells, '28).

Marshall and Crane, and H. L. White and his co-workers agree in placing urea and phosphates in an intermediate class whose members, though affected by volume, are not so closely related to it as chloride. This is confirmed by our figures for urea, which show a significant correlation with volume but one approximately only half as great as that between volume and chloride. But, from our data, which are confirmed by the observations of Bock and Iversen ('21, '22), phosphates, with a volume correlation of −0.08, fall rather in the third class recognized by these investigators (Marshall, White) of substances not affected by volume. In this class they also place creatinine and ammonia. The latter is certainly independent of volume according to our data; and although creatinine shows a fairly high correlation with volume (+0.31) in our 24-hour urines, in the short-period samples, which more nearly correspond with those studied by Marshall and by White, its independence of volume (+0.12) is apparent. It is not necessary to discuss here the significance of these findings for rival theories of kidney function; this confirmation (on the whole) by an independent statistical method of previous experimental findings gives added interest, however, to the discussions which may be found in the papers referred to (see also Marshall and Crane, '24).

Turning to the correlations among the organic constituents of the urine for evidence of metabolic inter-relationships, three substances, ammonia (excepting its correlation with urea), creatine and sugar (reducing substances) may be dismissed with little comment, as having coefficients either so small or so unlike in the two kinds of urines as to indicate almost complete independence in their origin and excretion. Exceptions to this which may be worth noting are the fairly high correlation between ammonia and urea, just mentioned, and the inverse relationship between creatine and creatinine, which, though low, is similar in both the 24-hour and short-period urines. Too little is known of creatine and creatinine metabolism to do more with this latter fact than record it as a statistical constant.

Correlation between ammonia and urea (or, total nitrogen; notice that the correlations between ammonia and the other nitrogenous components of the urine, amino acids, uric acid, creatinine, are insignificant) has long been known. Hasselbalch ('12, '16) and later Raffin ('26) have claimed that there is a constant ratio between the two at a given pH. Hubbard and Munford ('22) and later Hubbard have revised this to the extent of showing that the highest correlation is between urinary acidity and ammonia concentration, rather than total output—a fact which is taken to favor the renal origin of ammonia for neutrality regulation (Nash and Benedict, '21; Benedict and Nash, '29) as against its formation in the tissues, generally (Bliss, '26; Artom, '26). More recently, however, Polonovski and Boulanger ('28), on the ground of lack of constancy for the Hasselbalch and the Raffin formulae, have objected to the whole idea of the exclusive restriction of ammonia for regulating urinary reaction and contend that its elimination is much more complex than would be the simple result of an acid-base regulating function of the kidney, being complicated, if in no other way, by the preformed ammonia of the blood, no matter how small.

In this connection it may be recalled that Bourquin ('24) found that when the output of urea is increased in caffein diuresis there is an accompanying increase in the excretion of ammonia. Here the correlation could hardly be an artefact due to the simultaneous need of ammonia for acid neutralization, since caffeine produces a copious flow of gastric juice especially rich in HCl (Sollman, '18, p. 217) which, of itself, should lead to an alkaline urine. Also, it is well known that in liver disorders the output of ammonia is increased, since it "is not so completely transformed into urea before excretion" (Hawk and Bergeim, '26, p. 726). And, finally, although the demand for ammonia for neutrality regulation might run pari passu with protein catabolism and urea formation, it will be remembered that the correlation between ammonia and the other products of protein metabolism, amino acids, uric acid, creatinine, is negligible; suggesting a specific relation-

ship with urea. Also, it is worth noting that the correlation in the 24-hour urines (+0.49) is slightly more than twice as great as that in the short-period, basal ones (+0.23). This might suggest that during periods of exogenous increase of protein metabolism, when the concentration of protein split-products in the blood is high, ammonia fails to be quantitatively converted into urea and the two are excreted together; whereas under basal conditions there would be less opportunity for the escape of ammonia, except approximately that necessary for neutrality regulation, and so a lesser correlation with urea obtains.

To avoid misunderstanding it may be stated quite emphatically that no attempt is being made to disparage the importance of ammonia in neutrality regulation, but merely to argue, in so far as these data allow, for the possible appearance of ammonia (with urea) in the urine to some extent independently of this function. That a part of the ammonia (and, under basal conditions, perhaps most of it) is in the urine for this purpose may be inferred from the fact that aside from urea the only other significant correlation it has is with phosphates. This is in line with the parallel increase in phosphate and ammonia excretion that was observed by Campbell and Webster in night urines ('21) and urines of high acidity ('22).

Of the remaining correlations there is little to be said that would add to our understanding of metabolic origin or method of excretion. It may be seen that urea, creatinine and uric acid are closely correlated and that the latter, especially under the endogenous conditions represented by the basal urines, has its highest correlations with urea, creatinine and amino acids.

11. Correlations with total basal oxygen consumption

	+0.24	Oxygen, ammonia
Oxygen, urea	0.22	Oxygen, total nitrogen
	0.19	Oxygen, urea
	0.18	Oxygen, creatinine
Oxygen, total nitrogen	0.17	Oxygen, phosphorus
Oxygen, uric acid	0.14	
Oxygen, creatinine	0.11	

Oxygen, amino acid	0.08.....	Oxygen, amino acid
Oxygen, creatine	0.06	
Oxygen, ammonia	0.03.....	Oxygen, chloride
	0.01.....	Oxygen, uric acid
	—0.09.....	Oxygen, creatine

One of the chief interests in the work of which this is part was to define and, if possible, explain, day to day intra-individual variations of basal metabolic rate; and these urine analyses were primarily undertaken to discover what correlation might obtain between this and the prevailing level of protein metabolism as evidenced by urinary nitrogen. This has been referred to in the first report of the series (Griffith et al., '29, p. 616) where coefficients of correlation may be found for total and protein oxygen; the latter being derived from the total nitrogen figures of this paper. The accompanying tabulation (no. 11) gives also the correlation between total basal oxygen and the individual urinary constituents of which we have record.

While this work was in progress, Wishart ('28) brought out results dealing with the same subject, but more completely, in that the correlation was determined under conditions of wide variation of protein intake, as well as during the minor fluctuations of normal diet. The first gave large variations in both nitrogen excretion and basal metabolic rate in which the coefficients of correlation between the latter and total nitrogen, urea, ammonia, creatinine and uric acid ranged between +0.71 and +0.76; or, if a seeming lag of 3 days between nitrogen excretion and metabolic rate was allowed for, these coefficients rose to from +0.76 to +0.85. On normal diet, however, where errors of estimation are large in comparison with the actual changes in metabolism or excretion, the coefficients for metabolic rate and total nitrogen excretion were much reduced, being, for three subjects, between +0.41 and +0.15 and the latter scarcely greater than its probable error.

It is in this latter class that our results are comparable and confirmatory. Reference to our first report will show coefficients of correlation between basal oxygen and total nitrogen

of from +0.01 to +0.37 for the five subjects of our group, with a slightly higher average (+0.22) for short-period, basal than for 24-hour excretion (+0.17; see, also, tabulation no. 11, above).

Although therefore, the correlation between basal oxygen consumption and total nitrogen excretion is small when calculated from the day-to-day variations on normal diet, there is good reason to believe the soundness of Wishart's opinion that, under these conditions, the fundamental correlation is merely obscured by errors of estimation which then become large in comparison with the variations of the functions being determined. For it will be apparent in later sections when we come to deal with averages, as, for example, during menstrual or seasonal cycles, there is a close parallelism between the curves for nitrogen excretion and metabolic rate; i.e., the plus and minus errors of analysis cancel out and the correlated variations become more evident.

On account of its apparently close relationship to active protoplasmic mass, creatinine is the only nitrogenous excretory product, which, prior to the work of Wishart and of ourselves, has received attention as a possible index of the rate of cellular metabolism. Wishart's data and our own show that, as far as intraindividual variations are concerned, this substance has nothing to recommend it above urea or total nitrogen; and to this extent confirm the earlier negative findings of Palmer, Means and Gamble ('14) and Eichelberger ('25).

In conclusion, it is interesting to compare the correlation of oxygen consumption with excretion computed from analysis of 24-hour and short-period, basal urines, respectively. Thus, urea from either type of urine shows practically the same degree of correlation with the oxygen consumption; and the same, or rather similar lack of correlation, is evidenced by the remaining components of both urines, with the exception of total nitrogen and ammonia. The total nitrogen of the basal urines is correlated in higher degree with oxygen consumption than is that of the 24-hour samples; and this dif-

ference, though perhaps too small here to be of much significance, deserves attention as indicative of the much sharper contrast of the same type that will be apparent when we come to compare the seasonal variations of both functions. The most striking reversal, however, is shown by ammonia. There is no correlation between it and oxygen consumption and it stands the very lowest of the series when computed from analysis of 24-hour urines. On the other hand, its excretion in the short-period, basal urines shows higher correlation with oxygen utilization than any other component. Until this can be tested by further work, it is futile to speculate as to its meaning. If it is taken to indicate that variations in metabolic rate are related to fluctuations in acid-base equilibrium rather than specifically to variations in protein metabolism, then the lack of correlation with 24-hour ammonia excretion might be explained according to the argument of a preceding page which tended to show that the relation of ammonia to neutrality regulation is obscured in 24-hour samples by the operation of other factors. But its chief interest at the moment is as a challenge to further investigation.

II. THE EFFECT OF MENSTRUATION

The data from urines obtained during menstruation and during the first, second, third and fourth weeks of the intermenstrual period have been grouped and their averages are given in table 2. The grand averages for the three subjects are shown in figure 1.

Excepting creatine and uric acid (not included in the graph), all of the excretion products for which we have analyses appear to vary during the menstrual cycle according to one of the following two plans: 1) excretion lowest at about the time of menstruation and maximum during the intermenstrual period. Most of the substances belong to this group which includes: urea, creatinine, amino acids, total and undetermined nitrogen, sugar, water and, very probably, chlorides. The occurrence of maximum excretion during the intermenstrual period is well established by all of the indi-

TABLE 2

The effect of menstruation on urinary excretion. Averages of the determinations made during the menstrual periods and during the first, second, third and fourth weeks of the inter-menstrual periods. The data for E.F. and G.H. are derived from 24-hour, and those for K.L., from short-period, basal, morning urines. For purposes of comparison all are expressed as 24-hour values. The numbers in parentheses are the number of observations on which each average is based

FUNCTION	SUBJECT	MENSTRUAL PERIOD	WEEKS FOLLOWING MENSTRUATION			
			First	Second	Third	Fourth
Volume, cubic centimeter per 24 hours	E.F.	836 (7)	1026 (12)	907 (9)	1063 (10)	863 (10)
	G.H.	591 (7)	665 (11)	663 (9)	683 (10)	625 (9)
	K.L.	1921 (7)	2631 (20)	2424 (18)	2679 (18)	1752 (18)
	Average	1116 (21)	1680 (43)	2219 (36)	1728 (38)	1237 (37)
Specific gravity	E.F.	1.0236 (7)	1.0200 (12)	1.0202 (9)	1.0226 (10)	1.0205 (10)
	G.H.	.0260 (6)	.0256 (11)	.0282 (9)	.0243 (10)	.0259 (9)
	K.L.	.0131 (7)	.0106 (19)	.0141 (17)	.0114 (17)	.0188 (18)
	Average	1.0206 (20)	1.0172 (42)	1.0193 (35)	1.0179 (37)	1.0210 (37)
Urea-N, grams per 24 hours	E.F.	4.626 (7)	5.546 (12)	5.249 (9)	5.900 (10)	5.609 (10)
	G.H.	4.894 (7)	5.515 (11)	5.799 (9)	5.275 (10)	5.672 (9)
	K.L.	6.422 (7)	6.343 (19)	7.281 (18)	8.219 (18)	6.633 (18)
	Average	5.314 (21)	5.899 (42)	6.402 (36)	6.834 (38)	6.122 (37)
NH_3-N, grams per 24 hours	E.F.	0.509 (7)	0.477 (12)	0.444 (9)	0.505 (10)	0.495 (10)
	G.H.	.251 (7)	.321 (10)	.242 (9)	.288 (9)	.287 (9)
	K.L.	.197 (7)	.196 (19)	.198 (18)	.215 (18)	.261 (18)
	Average	0.319 (21)	0.309 (41)	0.269 (36)	0.311 (37)	0.330 (37)
Creatinine-N, grams per 24 hours	E.F.	0.370 (7)	0.391 (12)	0.380 (9)	0.404 (10)	0.365 (10)
	G.H.	.293 (7)	.334 (11)	.331 (9)	.325 (10)	.325 (9)
	K.L.	.357 (6)	.344 (18)	.354 (18)	.373 (17)	.372 (18)
	Average	0.339 (20)	0.355 (41)	0.355 (36)	0.368 (37)	0.358 (37)
Creatine-N, grams per 24 hours	E.F.	0.046 (6)	0.040 (12)	0.043 (9)	0.051 (10)	0.052 (10)
	G.H.	.046 (7)	.044 (11)	.053 (9)	.035 (10)	.051 (9)
	K.L.	.015 (5)	.027 (19)	.027 (16)	.021 (17)	.015 (18)
	Average	0.037 (18)	0.035 (42)	0.038 (34)	0.033 (37)	0.034 (37)
Uric acid-N, grams per 24 hours	E.F.	0.179 (7)	0.153 (12)	0.145 (9)	0.167 (10)	0.150 (10)
	G.H.	.109 (7)	.137 (11)	.121 (9)	.120 (10)	.136 (9)
	K.L.	.148 (6)	.156 (19)	152 (18)	.161 (17)	.141 (18)
	Average	0.145 (20)	0.150 (42)	0.143 (36)	0.151 (37)	0.142 (37)
Amino acid-N, grams per 24 hours	E.F.	0.100 (7)	0.111 (12)	0.135 (9)	0.113 (10)	0.106 (10)
	G.H.	.083 (7)	.099 (11)	.094 (9)	.093 (10)	.078 (9)
	K.L.	.088 (6)	.094 (19)	.094 (18)	.109 (16)	.089 (16)
	Average	0.091 (20)	0.100 (42)	0.104 (36)	0.105 (36)	0.091 (35)
Total N, grams per 24 hours	E.F.	6.210 (7)	7.094 (12)	7.063 (9)	7.583 (10)	7.140 (10)
	G.H.	6.147 (7)	6.902 (11)	7.163 (9)	6.801 (10)	7.005 (9)
	K.L.	7.613 (7)	7.859 (20)	8.369 (18)	9.559 (18)	7.953 (18)
	Average	6.657 (21)	7.400 (43)	7.741 (36)	8.313 (38)	7.503 (37)
Rest N, grams per 24 hours	E.F.	0.354 (7)	0.416 (12)	0.721 (9)	0.448 (10)	0.406 (10)
	G.H.	.470 (7)	.396 (10)	.486 (9)	.499 (10)	.468 (9)
	K.L.	.356 (6)	.351 (18)	396 (16)	.431 (16)	.407 (14)
	Average	0.396 (20)	0.382 (40)	0.506 (34)	0.454 (36)	0.423 (33)
Sugar, grams per 24 hours	E.F.	0.428 (7)	0.484 (12)	0.516 (9)	0.494 (9)	0.465 (10)
	G.H.	.468 (7)	.458 (11)	.690 (9)	.534 (10)	.512 (9)
	K.L.	.274 (6)	.344 (19)	.366 (18)	.366 (17)	.346 (18)
	Average	0.396 (20)	0.414 (42)	0.484 (36)	0.445 (36)	0.419 (37)
Organic acids, cubic centimeter, N/10	E.F.	581 (6)	558 (11)	581 (8)	573 (10)	558 (10)
	G.H.	442 (7)	373 (11)	457 (10)	418 (9)	375 (7)
	K.L.	489 (4)	481 (17)	482 (15)	542 (15)	464 (11)
	Average	502 (17)	472 (39)	498 (33)	518 (34)	475 (28)
Total acid, cubic centimeter, N/10	K.L.	138 (6)	104 (17)	119 (16)	172 (17)	209 (17)
Phosphorus, grams per 24 hours	K.L.	0.581 (6)	0.508 (17)	0.567 (18)	0.667 (17)	0.645 (18)
Chlorides, grams per 24 hours	K.L.	8.640 (6)	11.300 (18)	9.852 (18)	9.847 (16)	8.532 (18)

vidual curves, and, with infrequent exception, it occurs at the middle or during the latter half, so that the average curves of figure 1 are well authenticated in this respect. The apparent exception of chloride is probably not of serious import, since this is but the individual curve for subject K.L., for whom, alone, this was determined, and the established correlation between chloride and water would no doubt be evidenced in an average variation of the former, were more

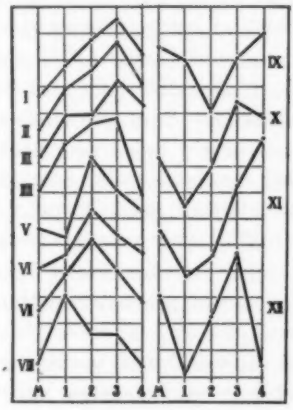

Fig. 1 The effect of menstruation on urinary excretion. The curves are derived from the grand averages of table 2. M, 1, 2, 3, and 4 refer to the menstrual period and the first, second, etc., weeks of the intermenstrual period, respectively. I, urea; II, total N; III, creatinine; IV, amino acid; V, undetermined N; VI, sugar; VII, volume; VIII, chloride; IX, ammonia; X, phosphate; XI, total acid; XII, organic acid.

data available, similar to the curve as we have it for the latter. The exact incidence of the lowest point is similarly uncertain. In most cases it occurs during the menstrual period, but there are enough instances of it coming just before or just after to make it impossible to say more than that it occurs at about the time of menstruation.

2) The second pattern of variation is represented by ammonia, phosphate, and total and organic acid. In these the minimal excretion occurs during the first half or middle of

the intermenstrual period and rises to a maximum toward its close. Again, individual differences prevent placing the maxima and minima with greater precision and it must be remembered that phosphate and total acid were determined on only one subject, K.L., so that the curves for these in figure 1 are individual and not averages. But, taken together, they show sufficient similarity to satisfy the expected physiological correlation and so strengthen and support one another.

Discussion. On the whole these results are in agreement with such previous work as we have been able to find. Like us, Rose ('17) and Stearns and Lewis ('21) were unable to find any connection between menstruation and the output of creatine. A retention of nitrogen during menstruation with a compensatory increase in output later was first reported by von Schrader (1894) and has since been confirmed by Murlin ('10, '11), Sherman, Gillett and Pope ('18) and Gillett, Wheeler and Yates ('19). Our results are not in agreement, however, with the report of Krause ('11) that after menstruation the distribution of nitrogen in the urine is changed so as to increase ammonia and undetermined nitrogen, while urea decreases. As shown above, we find this true only for the rest nitrogen, while urea increases and ammonia, though less certainly, decreases. And, although our data for ammonia are not too conclusive, the variation we have observed is similar to that previously reported by Bond ('22).

As regards water and chloride excretion, Heilig has reported ('24) that when water and chloride are ingested the amount excreted is only half as great during menstruation as during the intermenstrual period—a finding quite in accord with our result.

The failure of Sherman, Gillett and Pope ('18) and of Gillett, Wheeler and Yates ('19) to find a menstrual variation in the output of phosphates, deprives us of confirmation for the definite variation shown by our data. However, it must be remembered that these were obtained on only one subject and from basal, short-period, rather than 24-hour urines, which makes the result none too certain or too rigorously comparable to the previous findings.

In conclusion, it may not be inappropriate to call attention to concurrent variation of other functions which appear to fit consistently with the excretion described here and which by their interrelationship strengthen and support each other.

Thus, the basal metabolic rate, oral temperature (Griffith et al., '29 a), and pulse rate (ibid., '29 b) are, like the total nitrogen and most of its partition products, lowest during or just after the period and rise during the intermenstruum to their highest values just preceding the onset of menstruation.

At the time of our previous publication there was evidence for such a variation of the metabolic rate and it has since been confirmed by Matters ('29), Wible ('31), McClendon, Myrick, Conklin and Wilson ('31) and, although they refuse to so interpret their data, Sandiford, Wheeler and Boothby ('31). We were unaware at the time that our data merely confirmed previous findings of Cullis, Oppenheimer and Ross ('22) for temperature; and of these last and, also, Moore and Cooper ('23) and Truesdell and Croxford ('26) for pulse rate; and are happy to correct these unintentional omissions at this time.

Boothby and his co-workers have just been referred to as refusing to interpret the variations of metabolic rate which they have obtained as intrinsic menstrual effects. The ground of their contention is that the variations are small and no greater than might occur fortuitously. This is undoubtedly true and it is as true of any of the other variables, taken separately, as it is of the metabolic rate itself. It is even possible that the metabolic rate, pulse rate and temperature might vary concurrently but fortuituously, not as an intrinsic menstrual effect, but merely the reflection at the moment of the degree of relaxation, repose or apprehension prevailing at the time of the determination. It is difficult, or so it appears to us, to fit the data regarding nitrogen excretion into such a scheme of transient instability. Even the nitrogen of the short-period urines excreted during the time of the metabolism determination would hardly be affected by such conditions prevailing at the time; but the result was the

same with the 24-hour samples collected during the day preceding the metabolism determination. In both cases the nitrogen excretion is lowest during menstruation and highest toward the end of the intermenstrual period. And in the light of the correlation which Wishart, particularly, has established between nitrogen excretion and metabolic rate, the variation which now appears to be established for the latter would seem to be precisely as demanded.

To complete the picture, we may refer to the variation we have observed in alveolar carbon dioxid percentage and pressure, the blood carbon dioxid capacity and pulmonary ventilation (Griffith et al., '29 c and '29 d). These have been referred to in our preceding report (ibid., '29 d) and need not be discussed in detail here. Suffice it to repeat that the variation of alveolar carbon dioxid was the most clear-cut and indubitable of any menstrual effect we have observed; and in the preceding report it was shown that the expected physiological relationship obtained between it and the variations of alkali reserve and pulmonary ventilation. Finally, it may be added that ammonia, phosphate and total acid of the urine appear to be related logically with the alkali reserve, being excreted at a minimum (during the early part of the intermenstrual period) when this is highest. We have, then, in the alveolar carbon dioxid pressure, alkali reserve, pulmonary ventilation and acid excretion four independent measurements of the same fundamental physiological state, and their concurrent variation would seem to establish beyond all doubt the occurrence of deep-seated metabolic changes during the menstrual cycle. It is not unreasonable to hope that further work may establish a relationship between the metabolic group (oxygen consumption, nitrogen excretion, pulse rate and temperature), on the one hand, and these measures of the acid-base balance, on the other.

III. SEASONAL VARIATION

The conclusions of this section are based on the grand averages of table 3, which are shown graphically in figure 2. It may be recalled that four subjects (A.B., C.D., E.F., and G.H.) each furnished a 24-hour sample approximately once a week during 1925; so, for this year the grand averages for each month, on which the curves of figure 2 are based, are derived from approximately sixteen determinations apiece. During 1926, three subjects (A.B., C.D., and K.L.) furnished short-period, basal, morning urines usually twice a week, so the grand averages for this year are based on approximately twenty-four analyses per month.

In the discussion of these results, it seems necessary to distinguish between the 24-hour and the short-period, basal urines. For, with the exception of organic acid and possibly ammonia, which appear to vary somewhat in the same degree in both types, there is too little similarity between them to permit direct comparison. Thus, confining ourselves principally to total nitrogen, though what is said of it applies in varying degree to its partition products, there is no evidence that it undergoes any definite seasonal variation in the 24-hour urines. On the other hand, its variation in the basal urines is remarkably clear-cut and uniform, being maximum in the winter and (if we neglect the one irregularly low point in November) minimum in the early fall. And, in so far as they seem to follow any definite pattern at all, the same seems to be true of urea, creatinine, creatine, uric acid, amino acids, undetermined nitrogen, volume and chlorides. Phosphates are too irregular to permit any definition. And total acid (omitting the irregularly low point for October), organic acid and ammonia appear to vary according to a pattern that will be discussed in more detail in the following report and in which the lowest values occur in the spring and the highest in the late summer or autumn.

TABLE 3

Monthly averages. The data for 1925 are derived from 24-hour urines and are so given; those for 1926 were obtained from short-period, basal, morning urines and are all computed to values per 120 minutes which was approximately the average time of collection (see last entry of table 1). During 1925, each subject furnished one 24-hour sample approximately once a week; so the individual monthly averages are based on approximately four, and the grand averages on approximately sixteen determinations each. During 1926, each subject furnished, on the average, two short-period, basal, morning samples per week; so for this year the individual averages are based on approximately eight, and the grand averages on approximately twenty-four determinations each.

			VOLUME	SPECIFIC GRAVITY	UREA N	NH_3 N	CREATININE N	CREATINE N	URIC ACID N	AMINO ACID N	TOTAL N	REST N	SUGAR	ORGANIC ACID (0.1 N)	CORRECTED ORGANIC ACIDS (0.1 N)	TOTAL ACID (0.1 N)	PHOSPHATE	CHLORIDE
			Cubic centimeters and grams per 24 hours															
Total	1925	Feb.	864	1.023	5.99	.392	.428	.047	.162	.091	7.76	.68	.596	540.2	348			
average		Mar.	853	24	6.95	.488	.456	.043	.149	.095	8.59	.50	.573	486.0	287			
(A.B.,		Apr.	976	24	7.22	.399	.429	.062	.163	.110	8.95	.58	.545	498.4	297			
C.D.,		May	986	22	6.32	.410	.422	.052	.131	.086	7.83	.43	.541	425.5	319			
E.F.,		June	993	22	6.12	.411	.388	.051	.123	.108	7.62	.49	.438	505.3	329			
G.H.)		July	868	23	6.59	.390	.424	.042	.140	.118	8.45	.62	.470	581.5	404			
		Aug.	916	23	7.12	.435	.464	.051	.144	.135	8.87	.52	.537	592.4	386			
		Sep.	985	22	6.68	.450	.401	.058	.152	.096	8.23	.40	.510	521.1	339			
		Oct.	941	19	6.75	.427	.401	.028	.154	.082	8.29	.37	.610	538.8	362			
		Nov.	1058	22	7.04	.438	.472	.033	.180	.100	8.67	.41	.586	544.4	336			
		Dec.	945	26	7.31	.462	.437	.042	.182	.105	8.96	.44	.587	489.2	282			
	1926	Jan.	930	28	7.19	.473	.460	.035	.198	.110	9.14	.51	.587	508.4	292			
			Cubic centimeters and milligrams per 120 minutes															
(A.B.,		Feb.	157	1.016	668	33.4	40.7	2.2	18.2	9.7	810	44	33.3	44.3	29.3	9.91	46.4	1074.6
C.D.,		Mar.	211	13	700	25.1	39.2	2.9	15.0	8.4	804	49	44.7	39.6	22.0	7.77	42.1	1058.7
K.L.)		Apr.	169	15	677	26.8	37.1	2.8	15.4	7.8	774	46	32.7	39.3	22.3	10.65	46.6	1020.2
		May	191	13	670	26.5	37.9	2.1	16.2	9.2	784	45	33.3	42.7	21.4	9.76	46.0	1014.4
		June	207	13	638	29.0	37.8	1.9	15.1	8.1	772	49	30.2	37.7	20.8	11.83	46.0	980.5
		July	189	16	639	26.2	37.1	1.7	15.5	8.7	751	29	30.0	44.5	27.4	12.63	40.1	913.4
		Aug.	107	18	619	33.0	37.5	0.9	15.4	7.5	747	29	30.0	42.5	26.2	13.20	40.0	782.3
		Sep.	164	15	631	24.2	38.4	1.4	14.8	9.7	731	26	29.5	43.9	26.3	12.21	40.8	1012.5
		Oct.	186	15	625	27.4	38.9	1.0	15.5	9.0	735	31	30.0	45.7	28.0	4.84	42.8	1020.3
		Nov.	151	17	622	26.3	38.9	1.1	15.4	8.0	729	34	29.9	43.7	26.6	10.84	45.7	834.1
		Dec.	140	18	611	26.6	38.6	0.7	15.8	8.7	747	48	28.6	49.2	31.4	10.84	32.2	821.5
	1927	Jan.	139	20	650	31.3	38.7	1.6	16.4	9.0	788	41	28.6	50.3	32.6	13.88	30.3	844.8

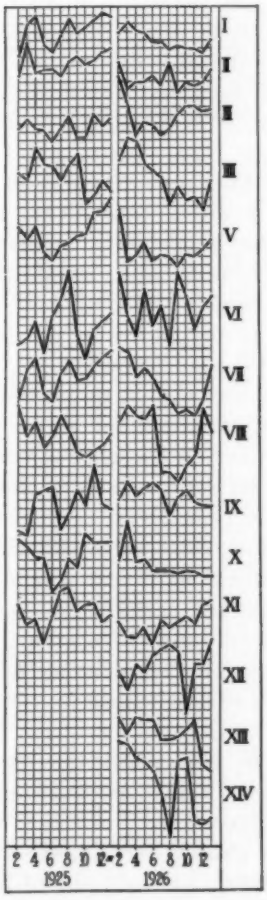

Fig. 2 Seasonal variation in urinary excretion. The curves are derived from the grand averages of table 3. Those for 1925 represent 24-hour and those for 1926, short-period, basal, morning excretion. I, urea; II, ammonia; III, creatinine; IV, creatine; V, uric acid; VI, amino acid; VII, total N; VIII, undetermined N; IX, volume; X, sugar; XI, organic acid; XII, total acid; XIII, phosphate; XIV, chloride.

Discussion. Denis and Borgstrom have reported ('24) that nitrogen excretion and therefore ingestion, of medical students in New Orleans, is lower than the average for colder regions; and, further that it is diminished in the warm as compared with the colder months. The first, which they regarded as giving probability to the second, has since been refuted by Youngburg and Finch ('26), Beard ('27) and Brooks ('29), who have shown that the average excretion of medical students is very similar in western New York, northern Ohio and North Carolina to that reported by Denis and Borgstrom for semi-tropical New Orleans; and, in addition, they have presented evidence seriously discrediting seasonal variation. Such, likewise, is the testimony of our own data for daily excretion, to which the above applies.

On the other hand, we know of no previous data comparable to that which we present here for short-period, basal urines and which we interpret as showing a very definite seasonal variation. These must represent very closely the endogenous metabolism; and it is a matter of very great interest that the type of variation which this shows (minimum in late summer or early fall) is identical with that which we have previously described for body weight, basal oxygen consumption (Griffith et al., '29 a) and basal pulse rate (ibid., '29 b). This must have the very considerable significance of indicating that the variation of metabolic rate which was characteristic of this group was not due to parallel variation in protein ingestion which would be chiefly responsible for the form of the curves for 24-hour excretion. Which is cause and which effect cannot at present be determined. All that can be said is that our data establish a closely correlated seasonal variation for basal nitrogen excretion and oxygen utilization.

An exception to the above is ammonia. As was mentioned previously, this, together with total and organic acid, seems to vary according to another plan which is widely represented among the physiological functions we have studied and which will be discussed in the following paper of this series.

SUMMARY

This is a continuation of reports already made (Griffith et al., '29) for basal metabolic rate and respiratory and cardiovascular functions in an attempt to define the variability and correlation of intra-individual variations over a course of time.

This report deals with the excretion of the principal nitrogen components of the urine, together with sugar, organic and total acid, phosphate and chloride.

Comparison is provided of the rate of excretion in 24-hour urines with that of short-period, morning urines under basal conditions.

By their coefficients of variation the relative constancy of excretion of these urinary components is established, 1) with respect to each other; and, 2) with respect to the other functions previously reported upon. As usual, the women of the group prove to be more variable than the men.

Coefficients of correlation are given which provide a measure of the interrelationship between, 1) the various urinary constituents themselves and, 2) between them and the simultaneous variations of basal metabolic rate.

Menstruation clearly affects the excretion of all these substances, with the exception, perhaps, of creatine and uric acid. Urea, creatinine, amino acid, total and undetermined nitrogen, volume, sugar and chloride are excreted in minimal amounts at about the time of menstruation and rise to a maximum in the latter half of the intermenstrual period. This is the type of variation previously observed in the women of this group for the oral temperature, pulse and basal metabolic rate. Ammonia and possibly organic and total acid and phosphate are roughly the reciprocal of this and show minimal excretion toward the middle of the intermenstrual period. This is shown to correlate with the previous findings in respect to menstrual variations in alveolar carbon dioxid pressure, blood alkali reserve and pulmonary ventilation. These correlations provide an integrated and more complete picture of physiological variation during menstruation than has been available hitherto.

There was apparently no seasonal variation for most of the components of the 24-hour urines. On the other hand, the total nitrogen and most of its partition products and volume and chloride of the short-period, basal urines are definitely excreted at a minimum during the late summer or early fall, with maxima in the winter. This is precisely the type of seasonal variation shown by the averages for this same group for body weight and basal pulse and metabolic rate. Exceptions to this type of variation are total and organic acids and, possibly, ammonia, which seem to be excreted at a minimum in the spring and maximum in the late summer or early fall, a pattern observed by many of the functions we have studied and which will be discussed in greater detail in the following report of this series.

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STUDIES IN HUMAN PHYSIOLOGY

VI. VARIATIONS IN BLOOD CHEMISTRY OVER LONG PERIODS OF TIME, INCLUDING THOSE CHARACTERISTIC OF MENSTRUATION

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TWO FIGURES

(Received for publication April 20, 1933)

Preceding reports of this series have described the intra-individual variation of basal metabolic rate, pulse rate and blood pressure, alveolar air and blood gas capacity, pulmonary ventilation (Griffith et al., '29) and urinary excretion (Pucher et al., '34) of five normal, adult subjects who were under observation during the 2-year period from February, 1925, to February, 1927. It is the purpose of this report to record observations on the composition of the blood of four of these subjects.

Two of the subjects, A.B. and C.D., were men and served continuously throughout the 2-year period; the other two, E.F. and G.H., were young women who were under observation during the first year (February, 1925, to February, 1926) only. For further details the first report of the series should be consulted.

From each subject, during the period of his or her service, 15 to 20 cc. of blood were taken from an arm vein approximately once a week. This blood was drawn, without disturbing the subject, and while in the basal state, either just preceding or just following the collection of the expired air for determination of the basal metabolic rate. For this pur-

pose the subject reported to the laboratory, without breakfast, between 8 and 9 A.M., and lay down. After a preliminary rest period of from 30 to 45 minutes the blood sample was taken, or the metabolism period run and the sample then obtained. In either event the blood composition may be taken to represent the basal condition.

As explained in the previous reports, during the day preceding the metabolism determination and, therefore, the taking of the blood sample, no meat was eaten. This single departure from otherwise unrestricted, normal conditions was made primarily to avoid any excessive stimulation of the metabolic rate by an occasional plethora of protein metabolites. Since, however, the subjects ate otherwise according to desire of milk, eggs and other meat substitutes it is hardly to be supposed that the nitrogen components of the blood would be abnormally affected.

The methods of analysis were standard and may be found most conveniently in the following citations from Hawk and Bergeim, Practical Physiological Chemistry, 9th edition:

Non-protein nitrogen; micro-Kjeldahl, page 368.
Urea nitrogen; aeration process, page 371.
Amino acid nitrogen; Folin process, page 380.
Uric acid nitrogen; Benedict method, page 377.
Total creatinine; page 376.
Sugar; Folin and Wu, page 381.
Cholesterol; Myers and Wardell modification, page 391.
Phosphate, inorganic; Fiske and Subbarow, page 403.
Calcium; Clark-Collip, page 408.
Chloride (Na); Whitehorn, page 401.
Corpuscles; Hematocrit.

I. STATISTICS

1. Averages. Table 1 gives the maximum and minimum, mode, arithmetical mean, standard deviation, coefficient of variation and number of observations for each blood constituent for each individual. Of these values, those for non-protein and urea nitrogen, sugar, calcium and chloride are within the usually accepted normal limits (Hawk and Bergeim, '26, loc. cit., p. 357); uric acid, total creatinine and

TABLE 1

Statistics of intra-individual variation of blood composition

FUNCTION	SUBJECT	MINIMUM AND MAXIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER OF OBSERVATIONS
Non-protein nitrogen. Milligrams, per cent	A.B.	21–38	28	30.0±0.3	3.72	12.4	91
	C.D.	20–37	27; 31	29.4±0.3	4.04	13.8	91
	E.F.	19–31	23; 24	23.9±0.3	2.73	11.4	46
	G.H.	20–40	29	28.6±0.4	4.29	15.0	43
	Average			28.0	3.69	13.2	271
Urea nitrogen. Milligrams, per cent	A.B.	7–18	13	12.5±0.1	1.97	15.8	91
	C.D.	6–17	11	11.2±0.2	2.28	20.3	95
	E.F.	5–12	7	8.2±0.2	1.98	24.2	46
	G.H.	8–17	9; 11	12.0±0.3	2.53	21.1	42
	Average			11.0	2.19	20.4	274
Amino acid nitrogen. Milligrams, per cent	A.B.	6.5–12.5	8.5	8.8±0.1	1.04	11.8	93
	C.D.	6.0–12.5	9.5	9.0±0.1	1.12	12.5	92
	E.F.	6.0–11.5	8.5	8.4±0.1	1.33	15.8	46
	G.H.	6.0–12.5	8.5; 9.5	9.0±0.2	1.58	17.6	43
	Average			8.8	1.27	14.5	274
Uric acid nitrogen. Milligrams, per cent	A.B.	0.6–1.4	0.9	0.96±0.01	0.17	17.5	90
	C.D.	0.8–2.3	1.3	1.32±0.02	0.22	16.6	93
	E.F.	0.7–1.6	1.0	1.13±0.02	0.19	16.4	46
	G.H.	0.6–1.7	0.9	1.05±0.02	0.23	22.3	42
	Average			1.12	0.21	18.3	271
Total creatinine nitrogen. Milligrams, per cent	A.B.	1.4–2.2	1.90	1.84±0.02	0.17	9.4	48
	C.D.	1.4–2.2	1.75; 1.80	1.77±0.02	0.19	10.5	50
	E.F.	0.9–2.3	1.80	1.78±0.02	0.23	12.9	47
	G.H.	1.4–2.4	1.80	1.88±0.02	0.17	9.1	45
	Average			1.82	0.19	10.5	190
Rest nitrogen. Milligrams, per cent	A.B.	1–14	6	6.60±0.2	2.52	38.2	87
	C.D.	3–16	7	7.20±0.2	2.67	37.0	88
	E.F.	1–15	5	4.60±0.3	2.99	64.9	46
	G.H.	1–10	3; 4	4.90±0.3	2.50	50.9	42
	Average			5.9	2.65	47.8	263
Sugar. Milligrams, per cent	A.B.	72–117	91; 92	89.4±0.5	7.37	8.3	92
	C.D.	70–117	103	99.5±0.8	11.90	11.9	93
	E.F.	73–116	93; 94; 95	93.3±0.8	7.62	8.2	46
	G.H.	79–127	97	100.8±1.0	9.47	9.4	41
	Average			95.8	9.09	9.5	272
Cholesterol. Milligrams, per cent	A.B.	70–130	110	106.9±1.0	12.20	11.4	90
	C.D.	70–155	115	112.7±1.1	13.40	11.9	90
	E.F.	85–135	110	109.1±1.1	10.40	9.5	44
	G.H.	95–150	110–120	116.2±1.1	10.50	9.1	42
	Average			111.3	11.62	10.5	266
Inorganic phosphate. Milligrams, per cent	A.B.	1.6–3.8	2.6	2.78±0.03	0.39	13.9	82
	C.D.	1.6–3.6	2.8	2.72±0.03	0.37	13.7	87
	E.F.	2.5–4.1	3.1	2.83±0.04	0.39	11.1	45
	G.H.	2.2–3.7	3.0	3.33±0.03	0.30	9.6	41
	Average			2.92	0.37	12.2	255
Calcium. Milligrams, per cent	A.B.	8.6–11.6	10.2; 10.4	10.19±0.04	0.59	5.7	81
	C.D.	9.1–12.3	10.3	10.32±0.04	0.57	5.5	87
	E.F.	9.0–11.9	9.9	10.10±0.06	0.56	5.6	44
	G.H.	8.9–11.2	10.2	10.23±0.06	0.53	5.2	39
	Average			10.22	0.57	5.5	251
Sodium chloride Milligrams, per cent	A.B.	455–545	475	489.2±1.7	17.45	3.6	81
	C.D.	460–610	475	496.6±1.8	24.75	5.0	84
	E.F.	455–550	475	499.1±2.7	25.25	5.2	40
	G.H.	475–565	495; 530	521.4±2.6	24.15	4.6	40
	Average			501.6	22.90	4.6	245
Per cent corpuscles	A.B.	37–54	45	45.7±0.2	3.12	6.8	92
	C.D.	42–55	46	47.3±0.2	3.03	6.4	90
	E.F.	37–48	42; 43	42.3±0.2	2.22	5.2	45
	G.H.	33–43	40	38.9±0.3	2.56	6.6	43
	Average			43.6	2.74	6.3	270

undetermined nitrogen, cholesterol and inorganic phosphate are slightly lower, whereas amino acid nitrogen is higher than the figures commonly given. Such differences as exist are, however, not large and (except the higher values for amino acids) may be attributable to the basal state, for which they are the most accurate definition at present available and as such perhaps hold whatever interest may attach to them.

2. Variability. One of the primary objectives in the study of which this report is the last, has been to ascertain the constancy with which the functions that have been investigated are regulated over a period of time. For this purpose we have employed the coefficient of variation and a summary of this datum for the blood constituents (taken from table 1) follows:

Coefficients of variation

Blood constituent	*Men*	*Women*	*Average*
Chlorides	4.3	4.9	4.6
Calcium	5.6	5.4	5.5
Per cent corpuscles	6.6	5.9	6.3
Sugar	10.1	8.8	9.5
Cholesterol	11.7	9.3	10.5
Total creatinine nitrogen	10.0	11.0	10.5
Inorganic phosphate	13.8	10.6	12.2
Non-protein nitrogen	13.1	13.2	13.2
Amino acid nitrogen	12.2	16.7	14.5
Uric acid nitrogen	17.1	19.4	18.3
Urea nitrogen	18.1	22.7	20.4
Undetermined nitrogen	37.6	57.9	47.8

A summary of the coefficients of variation for the functions previously reported upon, exclusive of those for urinary excretion, can be found in the fourth report of this series (Griffith et al., '29 d, p. 569) and for the excretion of the commoner urinary constituents in the preceding one (Pucher et al., '34). Reference to these will show that of twenty-seven measurements, including metabolic, cardio-vascular and respiratory functions, the coefficients of variation ranged from 0.5 to 12.9. Urinary excretion was uniformly less constant, varying, except for specific gravity (0.6) and creatinine under basal conditions (9.6), from 15.4 to 92.3. Objection could have been made that the higher variability of the uri-

nary constituents might have been due, at least in part, to greater error inherent in the methods of analysis involved in their determination. This suspicion is considerably allayed by the data given here for the blood constituents. The determination of these involved errors similar to those of urine analysis, yet, with the exception of urea, the greater constancy of the blood composition is apparent from the following summary:

Coefficients of variation

	Blood	*Urine*
Chloride	4.6	33.6
Sugar	9.5	22.9
Inorganic phosphate	12.2	29.4
Total nitrogen	13.2	16.6
Amino acids	14.5	28.0
Uric acid	18.3	25.5
Urea	20.4	18.8
Undetermined nitrogen	47.8	52.6

This is simply a statistical statement of a fact known long since that one of the important mechanisms for maintaining the composition of the blood within definite limits is variable excretion in the urine.

We know of only one previous attempt to define the intra-individual variability of the blood constituents; this is by Hammett ('20) who used the average deviation from the mean as the measure of variability. Our evidence is an almost exact confirmation of his, the only exception being that he found sugar more variable than creatinine or total non-protein nitrogen, whereas with us it is less variable than any of the nitrogenous components.

Another matter that has consistently forced itself upon the attention in connection with the functions previously reported upon has been the prevailingly greater variability shown by the women subjects of the group. Thus of the total of forty-two variables for which we have the data, the coefficients of variation were greater for the women than the men in thirty. It may be seen from the summary preceding the one given just above that this ratio does not obtain among the constituents of the blood reported on here. Of these only

amino acid, uric acid, total creatinine and urea are slightly, and undetermined nitrogen significantly more variable in the women than in the men.

3. Correlations. In conclusion, one of the objectives originally in mind when this study was undertaken was to determine the extent of day-to-day intra-individual variations of the basal metabolic rate and in how far these might be caused by, or at least correlated with simultaneous variation of other functions. In so far as the blood constituents reported on here are concerned there are no significant interrelationships between their variation and that of the simultaneously determined basal metabolic rate. Inspection of the correlation tables indicated in each case such complete lack of relationship that computation of the coefficients seemed an unnecessary expenditure of effort.

II. MENSTRUAL VARIATION

Blood samples were obtained from the two women subjects, E.F. and G.H., as a rule once a week during the year 1925. Data are available, therefore, for approximately twenty-four menstrual cycles. Those determinations made during the menstrual periods and each of the four weeks following have been grouped and the averages are given in table 2 and shown graphically in figure 1.

The only unequivocal variation shown by these data is by uric acid and chloride. Both are exactly alike with highest values during menstruation and a continuous fall during the intermenstrual period.

Except for the high value shown by E.F., in the fourth week, total creatinine would just as definitely follow the above pattern.

Also, except for the low value shown by E.F., during menstruation, cholesterol varies similarly in both subjects. The highest value is reached the week after menstruation and, granting the exception, the lowest 2 weeks later. Sugar, though more doubtful, is alike for both subjects, similarly lowest during the third intermenstrual week and probably highest at about the time of menstruation.

TABLE 2

Variation of blood composition during the menstrual cycle; averages of the observations made during menstruation and during each week following; the numbers in parentheses are the number of observations on which each average is based

FUNCTION MILLIGRAMS, PER CENT	SUBJECT	MENSTRUAL PERIOD	WEEKS FOLLOWING MENSTRUTAION			
			First	Second	Third	Fourth
Urea N	E.F.	7.67 (7)	8.22 (13)	8.50 (9)	8.01 (8)	7.96 (9)
	G.H.	13.21 (7)	12.44 (11)	10.37 (9)	12.64 (9)	11.97 (7)
	Average	10.44	10.15	9.44	10.46	9.71
Total creatinine N	E.F.	1.86 (6)	1.78 (13)	1.76 (9)	1.67 (9)	1.86 (10)
	G.H.	1.95 (7)	1.86 (10)	1.93 (9)	1.87 (9)	1.80 (9)
	Average	1.91	1.81	1.85	1.77	1.83
Uric acid N	E.F.	1.15 (7)	1.15 (13)	1.12 (9)	1.12 (8)	1.06 (9)
	G.H.	1.20 (7)	1.10 (10)	1.00 (8)	1.03 (9)	0.90 (7)
	Average	1.18	1.13	1.06	1.08	0.98
Amino acid N	E.F.	9.01 (7)	8.40 (13)	8.21 (9)	7.82 (8)	8.56 (9)
	G.H.	8.72 (7)	9.36 (11)	8.76 (9)	8.94 (9)	8.66 (7)
	Average	8.86	8.86	8.49	8.41	8.60
Total non-protein nitrogen	E.F.	23.1 (7)	23.4 (13)	24.5 (9)	25.0 (8)	23.3 (9)
	G.H.	30.9 (7)	28.4 (11)	27.2 (9)	29.2 (9)	28.0 (7)
	Average	27.0	25.7	25.8	27.2	25.4
Rest nitrogen	E.F.	3.4 (7)	3.9 (13)	4.9 (9)	6.4 (8)	3.9 (9)
	G.H.	5.5 (7)	4.7 (11)	5.2 (9)	5.0 (9)	4.7 (7)
	Average	4.4	4.3	5.1	5.7	4.2
Sugar	E.F.	92 (7)	92 (13)	95 (9)	90 (8)	95 (9)
	G.H.	103 (6)	101 (11)	101 (9)	95 (8)	104 (7)
	Average	97	97	98	93	100
Cholesterol	E.F.	102 (7)	113 (12)	110 (8)	108 (8)	110 (9)
	G.H.	119 (7)	123 (10)	116 (9)	109 (9)	114 (7)
	Average	111	117	113	109	112
Inorganic phosphate	E.F.	3.23 (6)	3.36 (13)	3.35 (9)	3.45 (8)	3.21 (9)
	G.H.	3.31 (7)	3.16 (10)	3.03 (9)	3.05 (8)	2.99 (7)
	Average	3.27	3.28	3.19	3.25	3.12
Calcium	E.F.	10.19 (7)	9.95 (12)	9.98 (8)	10.00 (8)	10.41 (9)
	G.H.	10.23 (6)	10.35 (10)	9.81 (9)	10.39 (8)	10.33 (6)
	Average	10.21	10.13	9.89	10.20	10.38
Sodium chloride	E.F.	506 (6)	500 (12)	503 (8)	498 (7)	486 (7)
	G.H.	526 (6)	524 (11)	520 (9)	522 (8)	514 (6)
	Average	516	512	512	511	499
Volume per cent, corpuscles	E.F.	41.1 (6)	42.5 (13)	43.7 (9)	42.2 (8)	41.4 (9)
	G.H.	40.0 (7)	38.6 (11)	37.8 (9)	38.9 (9)	39.0 (7)
	Average	40.6	40.7	40.7	40.5	40.4

Allowing for the paucity of the data and its rather arbitrary division into weekly averages all of the above four, uric acid, total creatinine, cholesterol and chloride, may not improbably vary according to a single pattern, with the highest values occurring at about the time of menstruation and the lowest toward the close of the intermenstrual period.

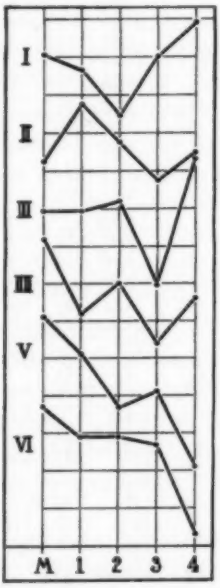

Fig. 1 Menstrual variation from the averages of table 2. M 1, 2, 3 and 4 are the menstrual period and the weeks following. I, calcium; II, cholesterol; III, sugar; IV, total creatinine; V, uric acid; VI, chloride.

Calcium is definitely lowest midway between periods.

The remaining substances, total and undetermined nitrogen, urea, amino acids, inorganic phosphate and corpuscular volume, are peculiar in that the curves for the two subjects are, in nearly every case, quite exact reciprocals of each other; so that although either subject, alone, may present a smooth curve of variation, the two are so exactly opposed that an average is unjustified and no conclusion possible.

For the sake of completeness, changes in blood-gas capacity which were described in the third report of this series (Griffith et al., '29 c) may be again referred to here. The blood oxygen and carbon dioxid capacity varied similarly, both being lowest just preceding or during menstruation and rising to a maximum during the first week following.

Discussion. That cholesterol may vary in concentration under the influence of menstruation is indicated by the finding of Guillaumin and Vignes ('28) that premature onset of menstruation is accompanied by increased cholesterolaemia and delay in menstruation by decreased cholesterol concentration. Efforts to define more precisely the cholesterol variations in normal menstruation have been made with particular thoroughness by Okey and Boyden ('27) who also review the previous work on the subject. This had been inconclusive in that according to one report blood cholesterol is highest preceding and during the menstrual period, followed by a rather sharp drop to normal; and according to another, high cholesterol values are followed by a fall immediately before menstruation, the lowest point being reached toward the end of the period, after which there is a rise followed within 2 days by a fall to the normal level which is maintained for 2 weeks. The results of Okey and Boyden, themselves, were more in harmony with the latter, consisting, as they report, of a premenstrual rise, a menstrual fall, a post-menstrual rise with a secondary fall to the normal level, the whole cycle requiring 2 weeks or more for completion. This, also, is the type of change to be observed in our data; so it would seem to be fairly established that the blood cholesterol values rise during the week following menstruation and then decline. This decline is arrested in the third week following menstruation when the concentration begins again to increase.

Cyclic variations in the blood sugar level must still be regarded as uncertain. Okey and Robb ('25) in the latest and most careful work on the subject concluded that there were none, except in the sense that the blood sugar level is more variable during the menstrual period than at other times.

This would be the conclusion, also, from the mutually contradictory results of previous work which they review. Both of our subjects are in agreement, however, in showing the lowest average values during the third week following menstruation. This is followed by a sharp pre-menstrual rise to well above the menstrual level. The only point in doubt from our data is the period immediately following menstruation. In one subject (E.F.) there is a post-menstrual rise, culminating the second week following menstruation and then dropping sharply to the low level of the third week. With the other subject (G.H.) the level is fairly constant, with only a slight fall, during the 2 weeks following menstruation and then falls sharply to the low level of the third week. Both are alike, however, it may again be repeated, in the sharp drop from the second to the third week and the equally sharp rise during the last part of the intermenstrual period to a pre-menstrual level higher than that during menstruation itself.

Although Okey and Robb failed to obtain evidence of a menstrual variation in the fasting blood sugar level, they were led to believe in a menstrual variation in carbohydrate metabolism from the observation that sugar tolerance curves resulting from the ingestion of 1.75 gm. glucose per kilo body weight showed lower maxima and a more pronounced secondary hypoglycaemia during menstruation than either just before or just after the period. This is what might be anticipated from the variation shown by our subject E.F., and the average curve in which the fasting level is higher just before and just after than during the period itself. It would be interesting to have tolerance curves for the interval at about the third week following menstruation during which both of our subjects showed the lowest fasting values.

The failure of our data to reveal any menstrual variation in blood inorganic phosphate confirms previous findings of Close and Osman ('28). Our figures, however, are rather to be described as uncertain than as positively either for or against a menstrual variation, in that each of our two subjects showed a well-defined cycle; one, however, being almost

exactly the reciprocal of the other. A similar lack of consistency characterized the findings of Okey, Stewart and Greenwood ('30) who were able only from a composite curve to arrive at a cautiously tentative suggestion that inorganic phosphate is lowest preceding menstruation and highest just after. Curiously, the average curve for our two subjects gives a quite similar result. The conclusion that such a variation is typical must wait, however, upon the collection of sufficient data to render such an average statistically sound or upon the demonstration of sufficient concurrence in individual cases.

In respect to blood calcium, Okey, Stewart and Greenwood (loc. cit.) summarize the best substantiated work preceding their own, as indicating slightly higher levels just before menstruation with a lowering during the period itself. Their own results, on the other hand, were the exact opposite of this and indicated a "tendency to frequency of low values a few days previous to the onset of menstruation and to frequency of higher values from the 8th to the 15th days following the onset of menstrual bleeding." Our own data tend rather to corroborate the earlier findings. Both subjects are quite alike in having the lowest values midway of the intermenstrual period, from which there is a rise to a maximum preceding the onset of menstruation itself.

The menstrual variation of serum chloride seems to be sufficiently clear-cut and definite to have led to complete unanimity in the preceding reports of it. Eisenhardt and Schaefer ('21), Close and Osman (loc. cit.) and Okey ('28) have all found it highest during the menstrual period; and this is in agreement with our data, in regard to which both subjects agree more closely than they do for any other of the blood constituents except uric acid and creatinine.

Non-protein nitrogen. The most thorough and painstaking study of variations of the non-protein nitrogen of the blood during menstruation is to be found in the previous work of Okey and Erikson ('26), which they have recently amplified and subjected to a very careful and critical analysis (Erikson and Okey, '31). In the first report they defined a menstrual variation of blood uric acid, which reached a high value immediately preceding menstruation amounting to 106 per cent of the intermenstrual level; this was followed during menstruation itself by a fall to a value of 86 per cent of the intermenstrual level; and was succeeded at or shortly after menstruation by a second rise to 105 per cent of the intermenstrual level. In their second report the pre- and post-menstrual rises and the menstrual depression are confirmed, but from the composite curve which they reproduce these seem to be of no more significance than other irregularities in other parts of the cycle; and the general impression, ignoring minor fluctuations, is that the values are lowest during menstruation and highest midway in the intermenstrual period. Perhaps the most significant fact, according to either of their descriptions, is that the blood uric acid level is lowest during the menstrual period itself.

This is not the evidence of our data, although we probably say so with much temerity, as opposing our meager ninety or so observations on two women to the disproportionately overwhelming number of 563 determinations on forty subjects as reported by Erikson and Okey. We merely submit as a description of our findings and await further work to reconcile or explain the difference, that both of our subjects are in unusually close agreement in showing the highest uric acid values during or just following menstruation, with a continuous fall to the lowest point just preceding the onset of the next period.

In both of their reports Okey and Erikson describe creatine, creatinine, urea and amino acids as unaffected by menstruation; this has also been reported for urea by Close and Osman (loc. cit.) and is confirmed by our data, in which the

curves for the two subjects are quite exactly the reciprocals of each other. Wang and Dentler ('20) have similarly reported no regular variation in creatine and creatinine during the cycle; their average figures for creatinine, however, are 1.35 during menstruation and 1.25 for the intermenstrual period; with, of the 15 cases studied, 9 higher during the period, 5 lower and 1 no difference; so the point might be made of a suggestion here of higher values during the period. Their conclusion of no change for creatine, however, seems the only one justified by their data; for the menstrual and intermenstrual averages are, respectively, 3.16 and 3.24 and, of the 15 subjects, 7 were lower and 8 higher during the period. So that if their figures were combined to express total creatinine, as ours do, they would indicate the possibility of higher values during menstruation; which is, also, the testimony of our subjects. Confirming Okey and Erikson, our data are negative for blood amino acids.

Finally, Okey and Erikson have found the total non-protein nitrogen of the blood decidedly elevated during the menstrual period; a rise which they find paralleled only by the undetermined nitrogen. This conclusion, again, is based on the massive evidence of 600 determinations on forty-two subjects, which makes us very glad that although our data do not entirely confirm it, neither are we required to completely disagree. The point is that our two subjects, in this respect, are quite unlike. Their curves for total non-protein nitrogen are, as might be expected, very similar to those for urea; and these have been described in the preceding paragraph as very accurate reciprocals of each other. One of them, however, is in complete agreement with the findings of Okey and Erikson, having the highest values for total non-protein and rest nitrogen (and urea) during menstruation and the lowest toward the middle of the intermenstrual period. So, although our data, alone, are inconclusive, there is partial agreement with the much better substantiated work of Okey and Erikson; and it seems justifiable to conclude that an increase in the total non-protein and undetermined nitrogen is to be expected during the menstrual period.

Okey and Erikson have made some effort to identify the component of the undetermined nitrogen responsible for its menstrual variation. The only matter of concern here is their report that it must be some element of plasma and not of the corpuscles, since these latter showed no menstrual variation in volume (hematocrit index) or hemoglobin. Our own data on corpuscle volume confirm this, again in the sense that the variations shown by each of the subjects are so exactly the reciprocal of each other that no conclusion is possible from our data. Curiously enough this is at variance with evidence provided by these same two women in regard to blood oxygen capacity (Griffith et al., '29 c) in which the menstrual is definitely lower than the intermenstrual level. It may be mentioned that this is not the only instance in our data of variance between hematocrit and oxygen capacity determinations; in connection with seasonal variation, to be referred to later, they vary inversely with respect to each other.

In conclusion, the evidence in regard to urea would seem anomalous; as has been remarked, although our two subjects do not agree with each other in the variation they show in total non-protein nitrogen or urea, these two components vary with marked similarity with each subject, there being practically a one-to-one correspondence; and this, it would seem, is what might be expected since urea constitutes such a large percentage of the total non-protein nitrogen. Indeed, in our material, the correlation between variations of urea and total non-protein nitrogen is much more exact, for each subject, than that emphasized by Okey and Erikson between the latter and undetermined nitrogen. We are not questioning the latter because it is evident in our data also; we merely raise the point that with the total non-protein nitrogen so markedly affected, as Okey and Erikson found it to be, urea would be expected to vary also and in the same sense.

Blood gas capacity. Attention has been called to our previously published finding (Griffith et al., '29 c) that the blood oxygen and carbon dioxid capacities are lowest during men-

struation and highest early in the intermenstrual period. We sincerely regret that at the time this publication was made we were unaware of previous work by Close and Osman ('28) and Okey ('28) which showed that just before or during menstruation the serum bicarbonate or the alkali reserve is lowered. On the other hand we are not sorry to have an opportunity of referring again to this matter because the variation in the closely related alveolar carbon dioxid percentage and pressure deserves the widest publicity, as being the most regular and unequivocal menstrual effect we have observed; and it would seem to be a matter of such deep-seated significance as to deserve further attention in connection with the mechanism of normal or disturbed menstruation.

In the report preceding this (Pucher et al., '34) may be found a résumé of the integrated relationships in variation that seem now to be established for the menstrual cycle: the blood-carbon dioxid capacity, carbon dioxid pressure of the alveolar air, pulmonary ventilation and acid excretion, on the one hand; and temperature, pulse and metabolic rate and nitrogen excretion on the other. These constituted two groups within each of which the variables appeared to be related according to physiological expectation. It remains to consider the nitrogen excretion in relation to the level of nonprotein nitrogen; the former has been described as lowest during menstruation and rising to a maximum toward the close of the intermenstrual period; while the evidence available, as reviewed here, makes it very probable that the latter is highest during menstruation and, on the whole, follows a course the reciprocal of the nitrogen excretion. It would seem possible, therefore, to attribute the diminished excretion during menstruation to retention rather than to diminished ingestion; and the decreased metabolic rate occurring at this same time (menstrual period) to an intrinsic menstrual effect rather than to decreased protein intake. This is a conjecture, however, the merits of which can only be determined by further work.

III. SEASONAL VARIATION

During the first year of this study (1925) a blood sample was obtained from each of the four subjects, A.B., C.D. (men) and E.F., G.H. (women) approximately once a week; during the second year (1926) samples continued to be obtained from the two men at the same frequency. The variations in both years are sufficiently alike to justify combination of the data into composite curves, shown in figure 2, in which each monthly value represents the average of approximately twenty-four determinations (excepting creatinine, which was determined only during the first year and the curve of which is therefore based on approximately sixteen analyses per month). With these have been included, for the sake of completeness, the curves of blood oxygen and carbon dioxid capacity, derived from data of our third report (Griffith et al., '29 c).

The results may be summarized as indicating four types of seasonal variation:

(1) Lowest in the summer and maximum in the winter: chloride and carbon dioxid capacity.

(2) The reciprocal of the above; i.e., minimum in the winter and maximum in the summer: cholesterol, uric acid and undetermined nitrogen. Of these, cholesterol is the best established, although during the first year it showed a marked depression in the spring which would ally it with the following group. Uric acid is quite uncertain and had better be considered altogether doubtful until further data are available; it is included here only because if it has any seasonal variation it is more of this type than any other. Much the same can be said for undetermined nitrogen, although if the apparently anomalous low values for July and August are disregarded the remainder of the curve is fairly satisfying.

(3) Minimum in the spring and maximum in the late summer or fall: oxygen capacity, total non-protein nitrogen, amino acids, total creatinine, phosphate and, less certainly, urea. With the exception of urea, the evidence for this type of variation is very convincing. In our earlier report on

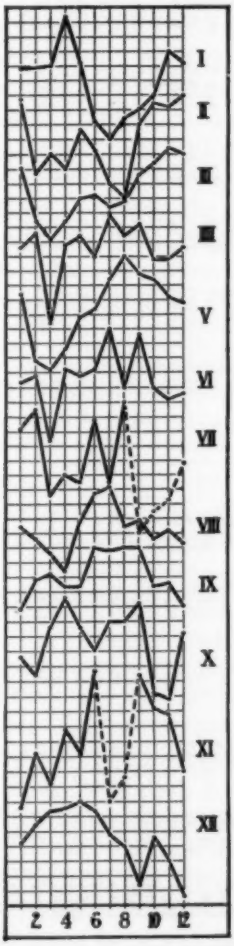

Fig. 2 Seasonal variation; 2-year averages from the data of table 3. From left to right, January to December. I, chloride; II, carbon dioxid capacity; III, oxygen capacity; IV, total non-protein N; V, amino acids; VI, total creatinine; VII, urea; VIII, inorganic phosphate; IX, cholesterol; X, uric acid; XI, undetermined N; XII, corpuscle volume.

TABLE 3

Seasonal variation in blood composition; grand averages. The data for 1925 are derived from four subjects, A.B. and C.D., men, and E.F. and G.H., women; during 1926 only the two men continued to furnish samples. A blood sample was obtained from each subject approximately once a week; each average is based, therefore, on approximately sixteen (1925) and eight (1926) determinations, respectively

SUBJECT AND DATE			BLOOD COMPOSITION—MILLIGRAMS, PER CENT											
			Urea N	Total creatinine N	Uric acid N	Amino acid N	Total N.P.N.	Rest N	Sugar	Cholesterol	Inorganic phosphates	Calcium	NaCl	Volume per cent corpuscles
A.B., C.D., E.F., G.H.	1925	Feb.	11.59	1.82	1.03	7.18	28.3	6.1	103.9	115.8	2.95	10.29	....	43.1
		Mar.	9.24	1.71	1.19	7.15	24.9	5.6	97.2	116.3	2.99	10.15	514.5	43.4
		Apr.	11.87	1.83	1.25	7.47	27.8	4.9	97.3	108.0	3.06	10.25	536.5	42.7
		May	11.52	1.82	1.26	8.24	29.2	5.5	93.6	103.5	3.14	10.13	524.8	42.8
		June	12.86	1.83	1.16	8.78	30.1	7.5	91.6	119.9	3.23	10.42	496.3	43.5
		July	11.35	1.90	1.21	9.67	27.2	3.1	96.3	118.8	3.26	10.17	481.3	42.5
		Aug.	12.56	1.80	1.27	10.99	31.5	4.3	99.9	118.8	2.81	10.15	484.5	42.2
		Sept.	10.37	1.89	1.22	9.69	28.9	6.7	97.8	116.5	2.91	10.34	484.5	41.8
		Oct.	10.63	1.80	0.96	9.69	28.7	5.8	93.0	109.2	2.81	10.16	494.3	42.4
		Nov.	10.58	1.78	0.95	8.39	26.1	4.3	93.7	105.8	2.98	10.32	507.3	43.3
		Dec.	10.92	1.79	1.09	9.01	26.9	4.3	93.8	105.5	2.99	10.08	496.3	41.9
		Jan.	11.48	1.81	0.97	9.22	28.4	4.9	96.6	108.1	2.91	10.03	511.8	43.7
A.B., C.D.	1926	Feb.	12.59		1.09	8.72	30.3	7.4	93.8	101.1	2.57	10.20	488.2	48.2
		Mar.	12.57		1.09	8.48	27.4	6.9	101.0	104.9	2.33	10.18	494.8	49.4
		Apr.	10.59		1.13	8.78	30.0	9.5	93.4	105.4	2.02	10.02	508.6	49.8
		May	10.70		1.01	9.16	29.1	8.1	97.9	109.7	2.64	10.32	488.9	50.1
		June	11.04		1.03	8.91	28.3	8.8	95.7	108.6	2.90	10.49	476.9	48.8
		July	10.86		1.08	9.03	29.8	8.8	95.8	107.6	2.97	10.35	479.7	48.2
		Aug.	11.47		1.03	8.58	28.3	8.5	98.6	110.4	2.88	10.04	491.4	47.8
		Sept.	10.56		1.13	9.19	29.4	9.5	98.3	112.9	2.86	10.03	495.2	45.6
		Oct.	10.85		1.09	8.97	30.4	9.4	94.5	105.3	2.71	10.15	495.5	48.2
		Nov.	11.14		1.08	9.66	30.7	11.2	88.5	108.6	2.66	10.67	514.2	45.6
		Dec.	11.82		1.17	9.02	29.9	8.7	92.7	93.6	2.46	10.52	515.6	44.8
		Jan.	12.18		1.22	8.89	29.2	6.8	90.6	88.7	2.77	10.94	496.3	46.5

the oxygen capacity we put more emphasis than now seems to us justifiable on the depression of the curve occurring during the summer and which would ally the variation of this function with that of the alkali reserve, as in (1) above; further consideration, however, inclines us definitely to the present classification.

(4) Finally, a single variable, the corpuscle volume, shows a type of change which is closely the reciprocal of that just described (3). In the previous section it was shown that oxygen capacity and corpuscle volume did not vary concurrently during the menstrual cycle; in that, whereas the former showed a definite variation, the latter was indeterminate; and it is but a further step from such lack of correspondence to the inverse relationship apparent here.

(5) Sugar and calcium have no recognizable seasonal variation.

It may be pointed out that types (2) and (3), above, have in common the occurrence of maxima at the same time of year; and that (4), being the reciprocal of (3), these might, not illogically all be regarded as effects of a common cause and, to that extent, fundamentally one type of change.

Discussion. We know of no previous literature on seasonal variation of blood constituents, except two reports on phosphate. Hess and Lundagen ('22) have described a variation of the blood of infants similar to that shown by our data; according to them, however, this was much less marked in older children and lacking in adults. We are confirmed in our result, however, by Havard and Reay ('25) who also obtained a variation in adults but with the minimum in January. It is not improbable, however, that the maxima and minima must depend on the conditions affecting the group studied; and the important point would seem to be the general agreement that the blood phosphate level is subject to seasonal change.

In conclusion, it may not be inappropriate in this last number of this series of reports to summarize the evidence which our data seem to reveal of seasonal variability in physiological activity. The backbone of this evidence is provided by

two functions which not only afford unequivocal testimony of seasonal variability, but which were capable of such direct measurement as to make it altogether improbable that the result is an artefact of analytical error; these are the basal pulse rate (Griffith et al., '29 b) and the volume of pulmonary ventilation under basal conditions (ibid., '29 d). According to the averages for our group, the first was lowest in August and highest in February; the second, lowest in April and highest in August–September. It will be noted at once and will be returned to later that, considering the period February–April as a unit, these are reciprocals of each other.

These two functions are regarded as fundamental, not in the sense of being considered the cause of any other variation that may appear, but rather as furnishing indubitable evidence that variation of two distinct types does occur. For, with respect to these two functions, all of the subjects of our group behaved alike during both of the years of our study. But the inference would seem sound that variation in these would be due to or accompanied by variation in other physiological processes.

Conclusions regarding other functions which give any evidence of seasonal variation are less certain than for the two just mentioned in that the individual curves are less regular and there is less exact correspondence between the maxima and minima of the curves for the different subjects; these are irregularities that might result from less direct and precise methods of measurement; or from the fact that the functions, themselves, are subject to subsidiary influences of unequal incidence with the various members of the group.

However, in practically all cases where there is sufficient concurrence among enough members of the group to give trustworthy evidence of seasonal variation in the average curve, this is found to belong to one or the other of the following two major patterns.

I

1. Basal pulse rate.
2. Body weight.
3. Basal metabolic rate; oxygen consumption per minute and calories per square meter per hour.
4. Basal excretion of water, chloride and nitrogen (excepting ammonia).
5. Blood chloride and carbon dioxid capacity.

II

1. Respiratory minute and tidal volumes with correlated variation in rate and the composition of the expired air.
2. Blood (a) oxygen capacity (and inversely corpuscle volume) (b) phosphate and cholesterol (c) nitrogen.
3. Basal excretion of total and organic acid and ammonia.
4. Oral temperature.
5. Basal and standing systolic blood pressure.

In spite of some scattering of the maxima and minima the first group may be said to represent a type of change in which the low values occur quite uniformly in August with high values in the period, February–March; in the second the high values are more evenly distributed over the period, August–October, and the low from March to May. It is not improbable that further work may necessitate some revision of this classification; or, granting indubitable seasonal changes and the general validity of this grouping, it is not unlikely that individual members of each of these groups may have really distinct maxima and minima and degrees of precedence as the seasons change.

However, for the moment and for the support thereby provided toward the establishment of these as bona fide physiological, rather than fortuitous variations, we are inclined to minimize the individual differences, as being due, perhaps in large measure, to insufficient data, and to emphasize the striking evidences of interrelationship which then become evident.

It is not possible to account for all the relationships within each group, but for the most part they are entirely rational. Thus, without any preconceived bias for or against seasonal variation in metabolism, the fact that weight, basal pulse and metabolic rate and nitrogen excretion all vary concurrently would seem strong proof that no one of them is merely accidental and fortuitous; likewise, if the blood chloride shows any variation it is not unexpected that chloride and water excretion would change similarly. Within the second group, the correlated variations of the respiratory functions have been commented upon previously (Griffith et al., '29 d) and need not be gone into in detail here; the variation of pulmonary ventilation appears adapted, also, to changes in acid-base equilibrium as shown by excretion of total and organic acid and ammonia; and, finally, most of the blood constituents vary similarly.

Likewise, conviction that we are dealing with a unified, organic response to changing conditions throughout the year receives support from the interrelationships between certain important members of each of the two groups. Thus, the variation in basal nitrogen excretion is evidently not due to changes in intake (Pucher et al., '34) so the evidence of an inverse relation to the blood nitrogen level provides the only other satisfactory explanation for the changes in both; and, finally, the alkali reserve is lowest just when the pulmonary ventilation and acid excretion are highest and vice versa.

From considerations such as these we feel confident, therefore, that a general, uniform variation in physiological activity is an established possibility; and this is all that we have been concerned to prove. Our opinion, the reasons for which were developed in the first paper of this series, that such variations as these are very probably not due to the operation of any one factor but express the integrated effect of many, would seem to have the virtue of reconciling such diverse results as have already been reported and to emphasize the need for extreme caution in generalizing from any one set of data. If, however, the possibility of cyclical variation is

accepted it follows that differences of the magnitudes involved must be allowed for in long-continued studies or in comparison of results obtained at different times; and interest is stimulated in the search for the causative factors that may be involved.

SUMMARY

This is the sixth and last of a series of reports dealing with intra-individual variation in a number of simultaneously determined physiological functions.

1. Statistics are given of the maximum and minimum, mode, mean, standard deviation and coefficient of variation for each of the following blood constituents: total non-protein, urea, amino acid, uric acid, total creatinine and undetermined nitrogen; sugar, cholesterol, inorganic phosphate, calcium, chloride and corpuscle volume.

2. Comparison of the coefficients of variation for the blood constituents and their urinary homologues (preceding paper) provides a statistical measure of the greater stability of the blood composition.

3. There is no correlation between the day-to-day, intra-individual variations of any of the blood constituents studied and similar variations in the basal metabolic rate.

4. There is evidence of menstrual variation as follows:

Cholesterol: rises during the week following menstruation; falls during the two following weeks; then begins to rise preceding the onset of the next menstrual period. Sugar and inorganic phosphate are doubtful but perhaps similar to cholesterol.

Calcium: highest during or just preceding menstruation and lowest midway of the intermenstrual period.

Chloride, uric acid and total creatinine: these show the most clear-cut cycles of any of the blood constituents, according to our data, and are highest during menstruation and lowest toward the end of the intermenstrual period.

Total non-protein, urea, amino acid and undetermined nitrogen: our data are doubtful but are in partial agreement with very trustworthy previous evidence for a cycle very similar to that for chloride, uric acid and total creatinine, above.

Blood-gas capacity (oxygen and carbon dioxid; from data in our third report) is lowest during menstruation and highest in the early part of the intermenstrual period.

5. There is evidence for seasonal variation as follows:

Chloride is lowest in the summer and highest in the winter; the same is true of the blood-carbon dioxid capacity (data from our third report).

Cholesterol, inorganic phosphate, amino acid, total creatinine, total non-protein and possibly, though less certainly, urea and undetermined nitrogen are highest during the summer or fall (July–September). For cholesterol and undetermined nitrogen the minima are in the winter (January); for the others it is during the spring (March–April). A similar variation is shown by the blood oxygen capacity (third report), while the corpuscle volume varies reciprocally, being highest in the spring and lowest in the fall.

Uric acid is very doubtful and calcium and sugar certainly give no evidence of seasonal variation.

6. By way of summary, attention is called to the evidence from our data of two major types of seasonal variation, roughly the reciprocal of each other: 1) Maximum in the winter and minimum in the summer; blood chloride and carbon dioxid capacity, urine volume, chloride and nitrogen excretion under basal conditions; weight and basal pulse and metabolic rate; 2) maximum in the summer or fall and minimum in the winter or (usually) spring: most of the blood constituents, as above; respiratory activity and urinary acid excretion under basal conditions.

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THE NUTRITIVE VALUE OF ANIMAL TISSUES IN GROWTH, REPRODUCTION AND LACTATION [1]

I. ALCOHOL-EXTRACTED BEEF LIVER

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INTRODUCTION

In most studies of growth and reproduction on meat diets, whole, raw, cooked, or cooked and dried tissues have been used. The biological differences shown to exist between the various tissues obviously reside not only in the protein, but also in the quality and quantity of other constituents. In order further to segregate these factors a series of experiments was planned in which the tissues that formed the source of protein were subjected to prolonged extraction with alcohol until fat-free. By the use of such materials the protein factor should more nearly be reduced to a single variable and any other important dietary contribution made by the tissue as a whole could be more clearly revealed. Beef liver was used in the present work and experiments with other tissues are in progress.

As early as 1917, Osborne and Mendel ('17) demonstrated improved growth resulting from small supplements of liver

[1] This research was supported in part by the National Live Stock and Meat Board through the committee on grants of the National Research Council. Progress reports have been presented before the Iowa branch of the Society for Experimental Biology and Medicine, Proc. Soc. Exp. Biol. and Med., 1931, vol. 28, p. 597; 1932, vol. 29, p. 669; 1932, vol. 30, p. 365.

[2] Doctor Smith had completed the notes for this paper a few days before his death. The data were somewhat reorganized with the assistance of Dr. H. A. Mattill. W.H.S.

to a lean beef diet, and their later experiments ('26) indicated that liver supplied the growth accelerating factors now commonly known as vitamins A and B. The extensive experiments of McCollum ('21) and his co-workers on the nutritive properties of animal tissues showed that 20 per cent of whole liver in the diet suffices as the sole source of protein for normal growth, reproduction, and rearing of young. Since the earlier work has been extensively reviewed in recent reports, especially by Clayton ('30), Daggs ('31) and Graham and Griffith ('27), detailed presentation is unnecessary at this time. Clayton, in her comparative study, investigated the relative efficiency of beef round, liver and kidney in both cooked and raw forms as sources of proteins and accessories, primarily for growth and reproduction. Qualitative and quantitative variations in the fat, as well as variations in the vitamin content of her diets, made the interpretation of the results as to the relative value of the proteins, as such, rather difficult, although kidney protein appeared to be superior, liver ranking next and muscle last.

EXPERIMENTAL

Animals. Albino rats obtained from the Sprague-Dawley Company, Madison, Wisconsin, were placed on experiment when they weighed 35 to 40 gm. They were kept in individual, wire-bottomed cages which were cleaned and sterilized at frequent intervals. Food cups were weighed and filled frequently so that the animals had access to food at all times. The animals were weighed every 5 days. The original lot was started in the first week of March, 1931, and later lots were started at approximately the same time of the year, in order to rule out seasonal variations between series, in so far as possible. Individual records, containing the growth curves, food consumption and breeding history of each animal, were carefully kept.

Rations. The rations were constituted as follows:

RATION	EXTRACTED LIVER	FAT	STARCH	PROTEIN PER CENT	ASH PER CENT	NUTRITIVE RATIO
A	18.7	15.0	60.3	15.0	4.3	1: 6.5
B	24.6	15.0	54.4	20.0	4.4	1: 4.6

Both rations: Agar, 2 per cent; salt mixture,[1] 4 per cent; cal. per gram, 4.6 (calc.).

[1] Osborne and Mendel salt mixture.

Hydrogenated cottonseed oil[3] was used to supply the fat of the rations, because it does not contain sufficient vitamin A to supply the needs of white rats, but does contain adequate amounts of vitamin E. Moreover, material of uniform quality could aways be obtained.

The fat-free liver, prepared by extracting beef liver, fresh from the slaughter house, with hot alcohol (Bloor, '26, p. 36) for 50 to 60 hours, had a protein content (N $\times$ 6.25) of 85 per cent and ash, 1.7 per cent. For calculating the composition of the diets, the remainder (13.3 per cent) was considered to be carbohydrate.

Preliminary experiments demonstrated that the diets were very deficient in vitamins A and B,[4] and these factors were supplied by the daily administration of 0.5 gm. of dried yeast[5] and 0.5 cc. of vitamin-tested cod-liver oil.

Growth. Growth records were kept for 120 days, although comparisons were finally made for the first 90 days only. Growth on the 20 per cent protein level (ration B) was normal or slightly above, whereas on the 15 per cent level (ration A) it was below normal (table 1). With few exceptions, the animals remained well and active for 250 to 275 days, although

[3] Crisco.

[4] In a paper on the nutritive value of liver, McHargue and co-workers ('30) present apparently quite different results. Their alcohol extraction of raw liver, which they do not describe, was doubtless less complete than ours, and their manner of preparing whole dried liver probably made it of as little value, so far as vitamins A and B are concerned, as our alcohol extracted liver.

[5] Kindly furnished by the Northwestern Yeast Company.

on autopsy it was not uncommon to find badly diseased lungs. Abnormally appearing organs other than this were rare.

Reproduction. All the females on the rations were fertile. The first matings were not controlled by vaginal smears, but these were begun when the animals were approximately 180 days old and after most of them had produced one or two litters. The estrous cycles at that time were irregular and marked by abnormally long periods of continued cornification and especially of resting stages. Later studies showed this to be the usual condition on the extracted liver diets. There was considerable difficulty in obtaining positive matings, in

TABLE 1

Gains in weight during 90-day period from weaning on extracted-liver rations

BASAL RATION	SEX	YEAST AND COD-LIVER OIL		YEAST, COD-LIVER OIL AND DRIED WHOLE LIVER (0.5 GM.)	
		1st generation[1]	2nd generation[1]	1st generation	2nd generation
		Gm.	*Gm.*	*Gm.*	*Gm.*
A	♂	186(8)[2]	162(7)	242(4)	231(4)
A	♀	148(10)	134(9)	171(4)	150(4)
B	♂	225(7)	199(6)	258(4)	277(4)
B	♀	162(8)	169(7)	172(4)	158(4)

[1] Includes series 1 (1931) and 2 (1932).

[2] Number of animals.

part due to incipient sterility in the males, but not entirely so, since the difficulty continued when vigorous stock males were used for matings. The testes of the experimental animals were normal in weight and the epididymes contained abundant, although very sluggish, sperm. Once positive matings were obtained, normal litters were cast. The abnormal cycles and difficulty in mating led to considerable loss of time, prevented an adequate reproduction history of the animals and limited the size of the experimental groups.

Lactation. Litters were reduced to six young within the first day or two after birth and weaned whenever possible, at 21 days of age. Lactation on the extracted liver diets was not entirely successful (table 2). In series 1 (1931), 54 and

51 per cent of the young born on rations A and B, respectively, were weaned. In series 2 (1932), carried through exactly a year later, only 23 and 22 per cent, respectively, of

TABLE 2

Lactation on extracted liver. First generation

RATION	SERIES	FEMALES	TOTAL LITTERS	TOTAL YOUNG	YOUNG WEANED	
					Number	Per cent[1]
A	1	3	9	59	30	54
A	2	3	9	69	14	23
					Av.	38.5
B	1	3	9	70	34	51
B	2	2	4	28	6	22
					Av.	36.5
A	Daily supplement of	3	9	71	29	48.3
B	0.5 gm. dried whole liver	3	9	75	23	30.7

[1] Weaning percentage calculated from the number of animals left after reducing size of litters.

TABLE 3

Lactation on extracted liver. First generation. Number and weight of young at weaning (21 days)

RATION	SERIES	FIRST LITTERS		SECOND LITTERS		THIRD LITTERS	
		Number of young weaned	Average weight	Number of young weaned	Average weight	Number of young weaned	Average weight
			Gm.		*Gm.*		*Gm.*
A	1	17	25.5	10	14.0	3	11.3
A	2	12	17.0	2	21.0	–	..
B	1	16	28.3	10	20.9	8	16
B	2	6	18.0	..	...	–	..
A	Daily supplement of	13	22.5	11	21.4	5	20
B	0.5 gm. dried whole liver	17	30.3	6	24.3	–	..

the young were weaned. First litter animals of series 1 were normal at 21 days, those of series 2 and the later litters of series 1 were definitely below normal weight (table 3). Although the weaning percentage was no greater on ration B

than on A, the higher protein level led to a better growth of the nursing young, suggesting better milk production. The difference in weaning between the two series, although unexplainable, is not a new experience. Clayton, for example, had weaning percentages of 39 and 8 in two lots of animals on her liver ration 130. Variation in the original animals undoubtedly is a factor and can only be controlled by the use of a larger number of animals from the same stock. The females usually lost weight during the lactation period; the losses averaged as much as 4 gm. daily and were generally less on the 20 per cent protein diet. It is impossible to state whether inability to nurse the young was due to scanty milk production, poor quality of milk or both. In most cases the milk band was apparent for the first day or two, but usually less distinct than one would expect. In many cases there were no visible signs of lactation.

Experiments to be reported later indicate that inadequate lactation was not due to a lack of any of the known vitamins, especially the vitamin B complex, which was furnished at a uniform, but perhaps minimal level to all animals recorded.

Second generation

Some of the second generation animals were continued on the diets supplemented with yeast and cod-liver oil. The growth of these animals (table 1) was inferior to that of the first generation, except for the females on ration B, whose growth rate approximated that of the females on the same diet in the first generation. The weaning weights of these particular animals were exceptionally high, which may account for their subsequent better growth. Since the young had a slow and varied growth rate, it was not possible to set an arbitrary weight or age for their removal. When they appeared to be able to eat for themselves, the most vigorous ones were placed on the experimental diets, usually at about 30 days of age. Their subsequent inferior growth rate as compared with that of the parent generation was probably due to the fact that their parents, the offspring of stock

colony rats, had been more heavily endowed during early life with certain necessary nutritive factors.

Reproduction was subnormal in the second generation. Estrous cycles were more irregular than in the first generation and it was even more difficult to obtain positive matings. At the end of a long period, some of the animals had borne only two or three litters and were in such poor physical condition that breeding experiments were discontinued. The males were almost entirely sterile, as shown by matings with normal females at estrus; testis weights were normal, but live sperm were seldom found in the epididymis.

Lactation was a failure in this generation. Young of nearly normal size were born, but most of them died within the first few days. In all, 26 litters, containing approximately 180 young, were born, and only 10 were weaned, with an average weight of 17 gm. at 21 days.

After certain animals had twice failed to rear their young, an attempt was made to restore normal lactation by a supplement of raw liver (1.5 gm. per day, equivalent to 0.45 gm. dry weight) beginning usually at the time of the placental blood-leak. In over half of these instances young were weaned, with weaning weights ranging from 25 to 40 gm. at 21 days. Apparently, raw liver furnished some factor, necessary for lactation, in which the basal rations were deficient, and which had not been supplied by yeast and cod-liver oil or by Harris vitamin concentrate or wheat germ oil of proved potency. Wilkinson and Nelson ('31) have reported that the addition of raw beef or hog liver to deficient diets decreased the mortality and increased the weight of the nursing young. Daggs ('31) showed that dogs on liver diets produced milk with a greater fat content than did those on diets containing other sources of protein; puppies nursed by mothers on liver diets grew faster. He suggests that liver is a better food for milk production, because "it may contain some hormone or lactation-producing vitamin in greater abundance. . . ." More recently, Mapson ('32) has demonstrated a favorable influence of raw liver upon growth and lactation in rats.

The occurrence in sources other than liver of unidentified substances favoring growth and lactation and which do not seem to be related to the known vitamins has also been suggested by Mason ('21) and Evans and Burr ('27) in lettuce, Coward ('29, '31) in casein and Sherman and his co-workers ('22, '24) in milk. Future developments in the study of the vitamin B complex may relate these substances to one or another of the B fractions, but this seems doubtful at the present time.

Simultaneously with series 2 (1932), a group of young animals was placed on the basal rations A and B supplemented with yeast and cod-liver oil, and also with ½ gm. of dried whole liver[6] daily. As is shown in table 1, these animals made considerably better gains than those not receiving whole liver. The most marked improvement was with the ration A animals. As judged by regularity of estrus, per cent weaning and weaning weights of the young (tables 2 and 3) the reproductive performance of these animals was considerably better than that of the simultaneous series 2 animals. Some of the young were continued on the same dietary (table 1, last column). The second generation male animals made more rapid gains than the females; later work has shown this to be the usual result of whole liver feeding. The animals were fertile, but lactation was not a success. Eleven litters were born to eight females but only three suceeded in rearing young, and these experiments were discontinued. Since dried whole liver was not as effective as raw liver in supporting lactation it was at first presumed that the lactation factor was different from the growth-promoting factor. Later experiments indicated that dried whole liver also aids lactation and that the difference is quantitative rather than qualitative. This is in line with the observations of Wilkinson and Nelson ('31) that the galactagog factor in liver is destroyed by drying the tissue at 120°C.

[6] Dried in a Buflovak, steam-heated, vacuum oven at 70 to 100°C.

Third generation

The animals of this generation were only those suckled by mothers that received the raw liver supplement and they were continued on the original basal rations, unsupplemented except for yeast and cod-liver oil. Owing to the impossibility of maintaining an adequate supply of alcohol-extracted liver about twenty animals having a similar growth-rate were discarded after 60 days on the rations. Table 4 therefore contains figures obtained both at 60 and at 90 days and shows that growth in the third generation was decidedly superior

TABLE 4

Growth on extracted-liver rations. Third generation

RATION	SEX	AVERAGE GAIN IN WEIGHT (GM.)	
		60 days	90 days
A	♂	172(11)[1]	212(2)
A	♀	124(9)	153(4)
B	♂	226(5)	249(2)
B	♀	142(3)	171(3)

[1] Number of animals.

to that of the second generation and slightly better than that of the first. From observations comparable to these, Mapson assumed the transmission of a growth-promoting factor. This was reflected in the breeding history of this group. The males were all fertile and the females showed less variation in estrous cycles, although positive matings were again hard to obtain. Normal offspring were produced and lactation was partially successful. In all nineteen litters were obtained and twenty-six young were weaned. This third generation, whose parents received supplements of raw liver, weaned 21 per cent of their young (based on elimination to six), as compared with 37 per cent for the first generation and 0 in the second before the addition of raw liver.

Fourth generation

Growth in this generation was comparable to that in the third. The males were fertile and normal litters were born. The animals were followed through two litters each and in all cases there was a complete failure of lactation. The effectiveness of the raw liver supplement, given the second generation animals, in improving the nutritional status of the third generation animals apparently extended in a small part to the fourth generation.

DISCUSSION

It is unlikely that the improved growth and reproduction following whole liver administration was the result of an increased intake of the vitamin B complex as this is ordinarily accepted. Since the protein in these rations was to be limited to that furnished by the extracted-liver, when additional amounts of the B complex were to be provided, the Harris vitamin concentrate was used. When administered in amounts six to ten times what is generally considered adequate this concentrate failed to restore lactation while 1.5 gm. of raw liver was strikingly successful.

Nor is it probable that the protein of the whole liver supplements was the effective factor since the feeding of whole liver produced an increased growth rate in animals on both rations. While those on the 15 per cent protein ration showed the more striking response, the consumption of 0.5 gm. dried whole liver or its equivalent daily, by each animal, increased the protein intake only to that of the animals on the 20 per cent ration. Furthermore it was determined by a limited number of experiments that the feeding of an equivalent amount of extracted liver to animals on ration B produced a negligible improvement.

Slonaker ('31) studied reproduction on various levels of protein and found a considerably higher weaning percentage, just as he did better growth, on a 14 per cent level of protein than on a higher level. He found a decrease in the size of the litters as the protein was increased, accompanied by a heavier

average weight of the young at birth. His basal diet contained 10 per cent protein distributed approximately equally between plant and animal sources and the higher percentages were obtained by adding meat scrap; the quality of protein was thus not uniform in all his rations. On our extracted liver diets in the first generation there were no significant differences in the weaning percentages on the two levels (table 2), or in the average number of young born per litter or in the average weight at birth (table 3). Supplementing the diets of the second generation with raw liver appears to have increased the average number per litter as well as the

TABLE 5

Influence of protein in the diet on number and weight of young at birth

Generation	1		2[1]		3		4	
Ration	A	B	A	B	A	B	A	B
Av. number per litter	7.0	7.5	6.7	5.7	8.5[2]	6.7[2]	7.1	10.0
Av. weight (gm.)	5.3	5.2	4.9	5.0	5.4	5.6	5.8	5.2
Ration, plus raw liver supplement								
Av. number			8.8	6.0	7.3	9.3		
Av. weight			5.5	5.5	5.2	5.8		

[1] Offspring of series 1.

[2] The mothers of these animals received 1.5 gm. of raw liver from the twelfth day of pregnancy through lactation.

average weight (table 5). The figures for the succeeding generations are variable and are less significant because of the smaller number of animals involved.

The vitamin E requirement was furnished by the 15 per cent of hydrogenated cottonseed oil in the rations. During the course of this work over 150 litters were born and there were no resorptions. The lactation deficiency was not a manifestation of a lack of E since an excessive amount of wheat germ oil was ineffective as a cure.

The irregular estrous cycles in the females and the impotence in the males can be correlated with the poor physical condition of the animals. The improved nutritive condition of the third and fourth generations was reflected in a greater

regularity of estrus in the females and increased fertility in the males. Evans and Bishop ('22) showed that quantitative undernutrition, produced by limited food intake, seriously interfered with sexual maturity and ovulation. Guilbert and Goss ('32) have presented a reproductive history of rats maintained on low protein (3.5 to 5.0 per cent) diets similar to that obtained on the extracted liver rations, a) irregular cycles, b) repeated failure of the females to mate at estrus when placed with normal males, and c) apparently infertile matings with recurrence of estrus. Irregular ovulation on heat-treated or alcohol-extracted casein diets has also been reported by Coward ('29, '31).

The removal of essential nutritive factors from animal foods by hot alcohol extraction appears to be an effective approach to the problem of identifying the tissue constituents with known or as yet unknown dietary requisites. The feeding of protein in such extracted form gives a more nearly true measure of its nutritive value. A study of other animal tissues is in progress.

SUMMARY

When alcohol-extracted liver furnished the protein of synthetic rations, all other recognized dietary requisites being supplied in uniform and presumably adequate amounts, growth of rats was normal at a protein level of 20 per cent; on a 15 per cent level growth was distinctly subnormal. In the second generation growth was below normal on both levels of protein. Lactation was deficient in the first generation and a failure in the second generation.

Supplementary feeding of dried whole liver increased the growth rate of first and second generation animals; raw liver supplements enabled the females to lactate and led to the weaning of more vigorous young, to better growth in the two succeeding generations, and to partial success in lactation in the third generation.

The reproductive mechanism was seriously deranged on these alcohol-extracted liver rations, as evidenced by irregular cycles and failure to mate at estrus.

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THE NUTRITIVE VALUE OF ANIMAL TISSUES IN GROWTH, REPRODUCTION AND LACTATION

II. THE PRESENCE OF A NEW DIETARY PRINCIPLE IN LIVER[1]

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TWO FIGURES

In the previous paper (Smith and Seegers, '34) it was shown that dried whole beef liver possessed a growth-promoting factor which alcohol-extracted liver had lost; this factor did not appear to be protein nor was it identified with any of the known vitamins. The reproductive capacity of rats on extracted liver declined in the first generation and almost disappeared in the second. Especially striking was the improved lactation secured by small supplements of raw liver added to a diet of extracted liver, and the advantage thereby conferred on the young (which did not themselves receive raw liver) was manifested by their better record of growth, reproduction and lactation as compared with that of their parents before these were given the raw liver supplement. These observations required confirmation and amplication, particularly as to the chemical nature of the growth and lactation factors and their possible identity with each other or with other already recognized food accessories.

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[2] Deceased.

[3] The experimental work reported in this paper was directed by Dr. H. Gregg Smith. The manuscript was prepared with the assistance of Dr. H. A. Mattill.

W. H. S.

The work described in this paper was designed first of all to demonstrate that the superiority of raw liver and of dried whole liver over extracted liver was real and not a fortuitous result. In order to reveal this advantage more definitely dried whole liver and alcohol-extracted liver were used, in the first series of experiments, not as small supplements but as the sole source of protein in otherwise identical diets.

Rations. With materials prepared as previously described and by the same experimental procedure (Smith and Seegers, '34) ration A containing 15 per cent protein in the form of alcohol-extracted liver was compared with a similar diet (ration C) containing the same amount of protein in the form

TABLE 1

	Ration A *Per cent*	*Ration C* *Per cent*
Alcohol-extracted liver	18.7	
Dried whole liver[1]		23.2
Hydrogenated cottonseed oil[2]	15.0	10.7
Cornstarch	59.8	61.6
Salts[3]	4.5	4.5
Agar agar	2.0	2.0

[1] Heated in a Buflovak, steam-heated vacuum oven at 70 to 100°C.
[2] Crisco.
[3] Osborne and Mendel as modified by Hawk and Oser ('31).

of dried whole liver. The composition of these rations is given in table 1. Except where otherwise stated, they were supplemented daily with 0.5 gm. of yeast[4] and 0.5 cc. of cod-liver oil.

Growth. Chart 1 shows the average gain in weight of six female and three male animals on each of the two rations (series 6 and 7), and of a like number on ration A supplemented with 0.5 gm. raw liver (series 8). Differences in growth response on whole liver were immediately noticeable; whether this was provided as dried whole liver (diet C) or as a supplement of raw liver to the extracted liver (diet A), growth was accelerated, particularly in the male animals. The total capacity for growth was also increased; the animals

[4] The yeast was kindly furnished by the Northwestern Yeast Company.

were heavier and more vigorous throughout life. After about 50 days those on the raw liver supplement began to lag behind those on the dried whole liver. This difference, which also continued throughout life, leads us to conclude, contrary to

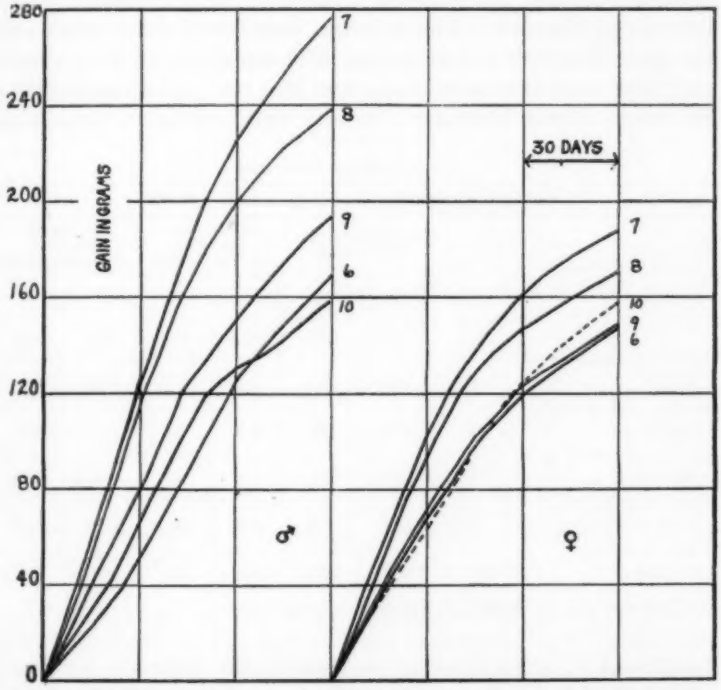

Chart 1 The curves represent the gains made by rats on the dried whole (7) and extracted-liver (6) rations, and the gains made when the extracted-liver ration was supplemented with raw liver (8), Harris vitamin concentrate (9), and a water soluble fraction of liver (10).

the statements of Mapson ('32) that the maximum effect of this particular growth factor in liver is not produced by that amount of it which is contained in 0.5 gm. raw liver. More than 0.5 gm. is necessary for optimum growth.

Food consumption. Records of food consumption have been kept for these series as well as for some 300 other animals

in the course of these studies. The amount of food ingested per gram increase in body weight has always been less when whole liver is fed. Figures for these three series and two others are given in table 2, and they clearly indicate a greater economy in the use of food and of its protein when whole liver is in the diet. The relation between these figures and the growth curves is interesting; the rapidly growing animals put more food into new tissue and less into maintenance than the more slowly growing. A note with special reference to

TABLE 2

Food consumed during 90-day growth period

DIET AND SUPPLEMENT	SERIES	AVERAGE GAIN IN WEIGHT		TOTAL FOOD PER GRAM GAIN IN WEIGHT		PROTEIN PER GRAM GAIN IN WEIGHT	
		♂	♀	♂	♀	♂	♀
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Diet A (extracted liver)	6	174	152	5.4	6.0	0.81	0.90
Diet C (dried whole liver)	7	278	189	3.8	5.2	0.57	0.77
Diet A + raw liver (0.5 gm.)	8	239	175	4.7[1]	5.4	0.79[1]	0.92
Diet A + Harris conc. (300 mg.)	9	187	152	5.9	6.1	0.80	0.86
Diet A + water fraction	10	158	162	5.9	5.9	0.81	0.85

[1] Including the raw liver supplement.

food intake and its relation to growth and efficiency quotients with paired feeding experiments was recently published ('32). While some workers have interpreted the growth-promoting effect of liver as a stimulation of the appetite we are inclined to favor the view expressed by Rose in the general statement ('28) that "when an added dietary component leads to an appetite stimulation the explanation is to be found, we believe, in the influence exerted upon the cells themselves."

Reproduction and lactation (table 3). In the breeding experiments females were mated with males on the same dietary. In series 6 (extracted liver) the estrous cycles were again

irregular and were marked by abnormally long periods of cornification and resting stages, with the latter predominating. With one exception after two litters were obtained from each animal on ration A reproduction was discontinued; from our experience the subsequent reproductive performance on this diet would have been unsatisfactory. Reproduction declined much more slowly in series 7 and 8 which received whole liver.

TABLE 3

Summary of reproduction

DIET AND SUPPLEMENT	SERIES	NUMBER OF FEMALES	NUMBER OF YOUNG BORN AND THEIR AVERAGE WEIGHT						PER CENT WEANED[1]	AVERAGE WEIGHT AT 21 DAYS
			1st litters		2nd litters		3rd litters			
			Number	Weight	Number	Weight	Number	Weight		
Diet A (extracted liver)	6	6	40	5.0	31	4.9	5	5.8	0	0
Diet C (dried whole liver)	7	5	51	5.7	38	5.7	22	5.7[2]	26.9	38.4
Diet A + raw liver (0.5 gm.)	8	6	43	5.8	44	5.3	26	5.4	34.0	19.5
Diet A + Harris conc. (300 mg.)	9	6	41	5.5	16	5.1	18	5.6	3.8	15.7
Diet A + water fraction	10	6	45	5.4	33	5.0	30	5.4	0	0

[1] Based on elimination to six, and is an average from all litters.

[2] Based on incomplete data.

From outward appearances all the young were normal at birth but their weight and number appear to have been influenced by the diet. The average number of young per litter in series 7 on dried whole liver was 8.4 with an average weight of 5.7 gm. per animal. Corresponding figures on the extracted-liver diet, series 6, were 6.3 and 5.1 gm. The results with the raw liver supplement, series 8, compare favorably with those of series 7. The precise reasons for the variations in the number and the weight of the young can only be determined by more extensive experiments. The number of young was doubtless influenced by the nutritional condition of the mother. Their weight may have been increased by the mother's immediate ability to lactate before the young were

weighed for the first time, and without specially devised cages it is impossible to determine the birth weight.

In comparison with earlier observations (Smith and Seegers, '34) the mortality of the young in these series was higher than expected, but such fluctuations are not uncommon and have also been referred to. Differences between series have at least been consistent. Lactation was a complete failure on the extracted-liver ration A. Both raw and dried whole liver enabled the females to nurse some of their young. Their weight at the time of weaning (21 days) was high compared to that of the young in the first series on ration A (Smith and Seegers, '34, table 3), where the protocols show a progressive decline in average weaning weights to as low as 11 gm. The marked difference in the weight of the young at the time of weaning clearly indicates that the growth response to liver feeding takes place early in life; and although the evidence is incomplete for reasons already stated, the response may even take place in uterine life. This is in part contrary to the observations of Mapson ('32) who believes that the time at which the growth response manifests itself in the liver-bred offspring is approximately that at which they begin eating for themselves. The inferior weight of the young, which mothers on the extracted-liver rations succeed in rearing, is a characteristic of the absence of the growth factor. The mothers lost weight during the lactation period, but this loss was no more than could be expected on a 15 per cent protein level (Kozlowska, McCay and Maynard, '32).

The difference in the nutritive value of the extracted and whole liver shows conclusively that alcohol extraction removes material from liver which is necessary for optimum growth and lactation. Since both diets (A and C) contained liver protein in equal quantities, it can be concluded that liver protein is not the influencing factor. This conclusion rests on the assumption that the extracted liver protein was not damaged in the process of preparing it. The validity of this assumption has experimental support in the showing made by series 8.

The lipid constituents of liver do not contain the active substance. Liver fat (ether soluble portion of alcohol extract) was substituted for the hydrogenated cottonseed oil in ration A to the extent of 6 per cent of the diet. This quantity of liver fat is approximately equivalent to the amount present in diet C. The growth of six animals was compared with that of six further controls on ration A. At the end of 60 days there was no difference between them and the experiment was terminated. That liver fat does not aid lactation is indicated by the fact that there was no improvement in lactation when liver fat (equivalent to 1.5 gm. raw liver) was given to female rats receiving basal ration A. This is in agreement with the conclusions of Wilkinson and Nelson ('31).

The possibility of the identity of the growth-promoting factor with the thermostable factors in yeast was given particular consideration. In the first of these experiments (series 9) 300 mg. of a yeast concentrate (Harris) was fed daily in addition to the 0.5 gm. of dried yeast which was the minimum daily allotment for all animals. The particular preparation used was tested separately and 60 to 75 mg. per day provided adequate vitamin B for growth. The use of this concentrate avoided a change in the dietary protein level. The records of these animals (chart 1, tables 2 and 3) contain only two indications that they derived any benefit from the concentrate. The males grew at a more rapid rate and the females succeeded in weaning about 4 per cent of their young. On the other hand, the females made only slightly better gains, had irregular oestrous cycles, and the few young which they reared were subnormal in weight at 21 days of age. Both sexes utilized their food uneconomically.

A watery liquid derived from the dehydration and extraction process (Bloor, '26, p. 37), in the preparation of the extracted liver, was tested for the factor here concerned. It was fed in $\frac{1}{2}$ gm. equivalents as a supplement to ration A. The records of the animals receiving this preparation, also given in chart 1 and tables 2 and 3, show that except for a

slight improvement in the growth rate of the females the animals did as poorly as the controls receiving diet A alone. Apparently the growth-promoting and lactation factor was destroyed in the process of preparing the extract, or partially destroyed and too small an amount was fed. Mapson's ('32) experiments indicate that the growth factor is water soluble, and our preliminary experiments with other preparations also indicate this.

The results obtained with vitamin concentrates are often confusing and difficult to interpret by various workers. For that reason and also because the several members of the vitamin B complex recently described appear to be present in yeast, studies were made of the effect of varying the amount of yeast. Chart 2, figure 1, shows the effect of supplements of ½ gm. and of 1 gm. of yeast to diet A. The resulting improvement can be ascribed, at least in part, to the yeast protein, for it was shown in the previous paper that a 15 per cent protein level did not give normal growth.

Figure 2 shows the result of a second experiment in which diet A and also C (15 per cent dried whole liver protein) were supplemented with 1 gm. of yeast daily. A marked difference was immediately noticeable; the whole liver produced its usual effects. The difference is particularly striking when one considers that the animals receiving diet C were at a disadvantage, because the value of the yeast protein was opposed by a diminishing increment in growth rate. Nevertheless the animals on the extracted liver made inferior gains. Beginning with the sixtieth day three animals received diet C in place of diet A. They responded to the change immediately and after 40 days, when they were discontinued, their weights were rapidly approaching those of the animals which had received ration C from the start. Their rate of growth was accelerated beyond that usually obtained on ration C at that age. This may be regarded as a resumption of growth after stunting and is a confirmation of the early work of Osborne and Mendel ('15).

In figure 3 of chart 2 are data which confirm and demonstrate more convincingly that yeast does not supply the factor which liver contains. Ten per cent of yeast was included in the basal rations A and C in place of an equal amount of

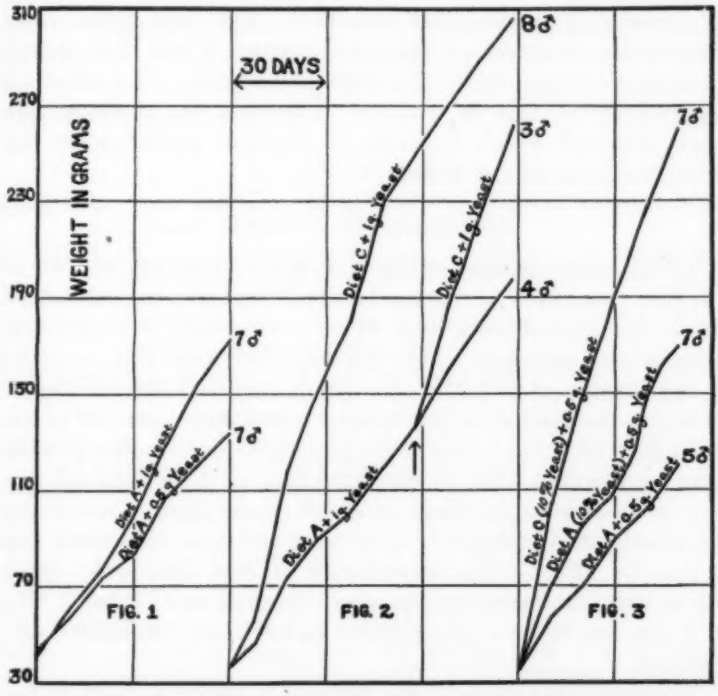

Chart 2 The chart shows the combined growth curves of male albino rats, and illustrates the effect of varying the amount of yeast fed with the extracted-liver ration A, and the dried whole liver ration C. Each animal received 0.5 cc. of cod-liver oil per day.

starch. In order to induce the animals to eat their daily allotment of cod-liver oil an additional ½ gm. of yeast was supplied as a daily supplement. The yeast employed was the dry product of the Northwestern Yeast Company, and portions from a number of shipments were used in these experi-

ments. If the liver factor is present in yeast it must be there in very small amounts, which could be revealed only by feeding yeast on a much higher level.

In the earlier work a special effort was made to use only freshly dried liver. As the work progressed it soon became apparent that this was not necessary. The dried whole liver used in the experiments recorded in chart 2 had been stored at ice-box temperature for a year and a half. The practical significance of this is obvious. Likewise, no evidence has been obtained which indicates a seasonal variation in the nutritive value of the tissue.

DISCUSSION AND SUMMARY

These observations confirm the conclusions arrived at in the previous paper and leave no doubt that alcohol extraction removes material from liver which is necessary for optimum growth and lactation. They further show that this material is not associated with the liver lipids and that the additional protein consumed when whole liver supplements are fed is not the effective agent. The principle which accelerates growth may be identical with the lactation factor; more than 0.5 gm. raw liver or its equivalent of dried whole liver is necessary for maximum response in each case, and heat treatment has a parallel effect. The superiority of raw liver over dried whole liver for lactation was also observed by Clayton ('30), and appears to be a quantitative rather than qualitative difference.

The multiple nature of the vitamin B complex leads to the easy assumption that the valuable contribution made by whole liver may be one of the several constituents of this complex. Though partially indirect, the evidence presented seems to differentiate this thermostable dietary factor from those present in yeast as well as from any of the hitherto known dietary essentials, and substantiates the conclusions reached by Smith ('31) in a preliminary report of this work.

Mapson ('32) has recently drawn similar conclusions from results obtained by quite different experimental procedures

and has tentatively proposed the name 'physin' for this factor. Physiologically improper proportions of the accepted dietary requisites are known to produce nutritional abnormalities and this new factor may yet prove to be a hypothetical substance, but the failure to obtain optimum growth and lactation on extracted-liver rations, when the known requirements are supplied in abundance, presents a contrast to the relatively small amount of whole liver which will restore these functions to normal.

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IRON METABOLISM STUDIES IN A NORMAL SUBJECT AND IN A POLYCYTHEMIC PATIENT

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TWO FIGURES

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This report presents the results obtained in a study over a period of 7 months of the total iron intake and output of two subjects, one normal and one suffering from polycythemia vera. During this investigation red blood cell counts and hemoglobin determinations were made twice a week.

The patients were confined to the Metabolic Ward of the Russell Sage Institute of Pathology where food was prepared under special precautions. Distilled water was used for cooking, drinking and final rinsing of utensils. All cutlery used was plated and all cooking utensils were aluminum or agate. Care was taken to avoid chipping of the agate. Aliquot portions of all foods were analyzed as described in a publication (Toscani and Reznikoff, '34) appearing in this volume, so that even canned foods were checked against possible iron contamination. Iron was determined by the Elvehjem ('30) and Kennedy ('27) methods and blood counts were done by the same individual with standardized pipettes. Fifteen and eight-tenths grams of hemoglobin per 100 cc. of blood was considered 100 per cent.

[1] The authors desire to express their thanks to the Misses Mabel Quinto, Janet Costello and Thelma Springer for their cooperation.

The urine and stools of the subjects were collected quantitatively and 4-day periods of the excreta were examined at a time. On a few rare occasions 3-day periods were used. In the case of the normal individual the shortest experimental phase was 2 periods; the longest 7; and the average 4. The shortest experimental phase for the polycythemic patient was 2 periods; the longest 12; and the average 5.

The normal subject was a middle-aged male who had come into the hospital complaining of diarrhoea and showed evidences of slight malnutrition and pseudopellagra. After several weeks in the regular ward he had completely recovered and when he was transferred to the metabolic ward was apparently normal. Table 1 and chart 1 give a composite picture of the results obtained with this normal subject.

A summary of the findings shows that this normal subject had a positive balance of more than 5 mg. of iron daily on an intake of about 26 mg. After parenteral liver therapy the retention was increased to 13 mg. daily. This positive balance persisted so that on a 19-mg. intake he stored 8 mg. daily. When he was placed on a 10-mg. intake he was practically in balance. Now parenteral liver therapy, on the same 10-mg. intake, gave him a retention of almost 3 mg. a day. After the possible effect of the liver wore off he again went on approximately 10 mg. ingestion and was in iron balance. On 17-mg. intake he showed storage of about 2.5 mg. daily. A liver extract, supposedly effective in secondary anemia (Whipple, Robscheit-Robbins and Walden, '30), containing a daily iron ration of 60 mg. and, when given with 17 mg. in food, seemed to give a retention of 17 mg. daily. A control period of 17.5 mg. alone gave a balanced condition. Iron alone, in the same quantities as the liver extract contained, added to that in the diet gave a retention of only 6.5 mg. When the patient was now placed on 80-mg. intake and parenteral liver extract was given he showed a rather surprising loss of about 30 mg. of iron daily. At this point it might be of significance that from the start of the experiment until the beginning of this negative phase the subject stored a total of 1388.6 mg. of iron.

TABLE 1

NUMBER OF PERIODS	AVERAGE DAILY IRON INTAKE (MG.)		AVERAGE DAILY TOTAL IRON EXCRETION (MG.)	AVERAGE DAILY IRON BALANCE (MG.)	BLOOD COUNT		COMMENTS
	Food	Medication			Hb, per cent (15.8 gm. = 100 per cent)	R.B.C. in millions	
					76	4.9	
5	19.4	7.0 (yeast)	20.7	+ 5.7	78	5.1	
					75	4.9	Lederle's parenteral
9	19.4	7.0 (yeast)	13.3	+13.1	73	4.4	liver extract, 9 cc. in
					86	5.0	first period
2	19.2		11.1	+ 8.1	81	5.0	
					84	4.7	
3	10.0		9.3	+ 0.7	92	4.8	
					104	4.8	
					100	4.6	Lederle's parenteral
7	10.1		7.3	+ 2.8	92	5.1	liver extract, 6 cc. in
					107	4.4	first period
3	10.8		10.9	— 0.1	96	4.9	
					83	4.2	
2	16.8		14.3	+ 2.5	90	4.8	
					99	4.6	
6	16.8	60.0 (liver extract)	59.8	+17.0	88	4.5	Lilly 55 liver extract
					90	5.0	daily
2	17.4		17.9	— 0.5	87	4.3	
					75	4.4	
4	17.2	60.0 (iron & ammonium citrate)	70.7	+ 6.5	82	4.4	
					86	4.9	
5	17.9	60.0 (iron & ammonium citrate)	107.9	—30.0	90	4.5	Lederle's parenteral
					81	4.0	liver extract, 9 cc. in first period
4	17.8	60.0 (iron & ammonium citrate)	86.1	— 8.3	69	4.4	$CuSO_4$, 15 mg. daily
					75	4.9	
3	19.1	2000.0 (iron & ammonium citrate)	1952.5	+66.6	81	4.2	3 gm. of NH_4Cl daily in
					96	5.0	last two periods

In connection with this apparent reversal of effect of liver extract it is interesting to note that Beckmann ('30) found that in secondary anemia liver caused a marked retention of iron. Riecker ('30) has pointed out that in a remission of pernicious anemia there is an increase in excretion over intake of iron. Therefore the effect of liver extract on iron balance depends to a considerable extent on the relation between tis-

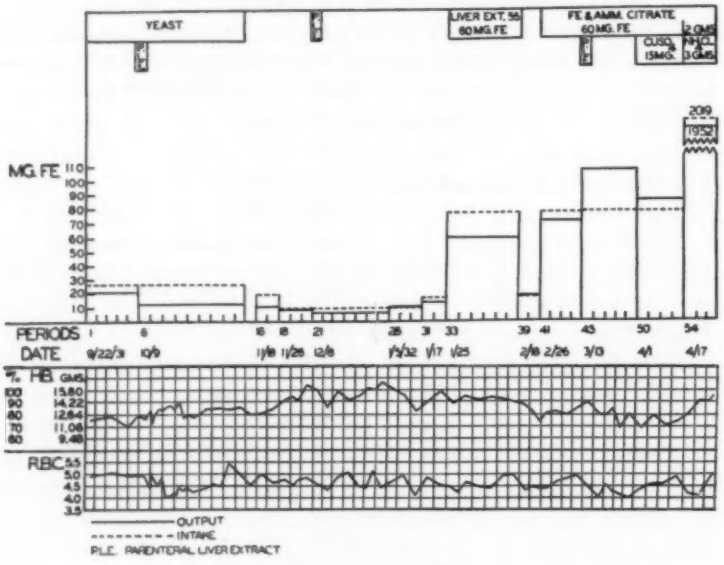

Chart 1. Normal subject

sue iron, hemoglobin iron and ingestion and excretion at any particular time, probably as Josephs ('32) indicates. Copper sulfate apparently cut down this iron loss to 8.3 mg. daily. Josephs ('32) found that copper has no effect on iron retention and tends to shift iron above a basal concentration from the tissues to hemoglobin formation. It is true in this experiment that during the copper administration there was some increase of red cell count and hemoglobin. It is interesting to note that massive doses of iron and NH_4Cl (Aub et al., '26)

had no effect on the urinary excretion of iron in view of Riecker's report ('30) of high concentrations of iron in the urine.

Red cell and hemoglobin studies in this normal individual seemed to show at first a striking independence of variations in iron intake. This is not surprising when one realizes that the storehouses for iron, even in anemia, have an ability to retain iron despite the apparent demands of the blood. However, it can be seen that adequate iron intake, 20 mg. daily, maintained a count of about 5,000,000 and a hemoglobin value of 85 to 100 per cent. Immediately after parenteral liver extract was given a short period of diminished hemoglobin and erythrocyte count was obtained. This is not infrequently seen in liver therapy in pernicious anemia. When the subject was placed on a 10-mg. intake his red cell count decreased to 4,200,000 and his hemoglobin to 83 per cent. This rapidly increased on a higher iron intake. After his period of marked negative balance his count again decreased to 4,000,000 and between 70 and 80 per cent hemoglobin. But on discharge after a $CuSO_4$ and a very high iron intake phase his final count was 5,000,000 R.B.C., 96 per cent hemoglobin. It is of interest to note that his initial hemoglobin value was 76 per cent of 15.8 gm. His total retention of iron throughout the experiment was 1413.2 mg. If converted entirely into hemoglobin he should have shown an increase in hemoglobin of 6 gm. or 38 per cent per 100 cc. of blood (Bassett, Killip and McCann, '31). This would have given a final hemoglobin reading of 114 per cent if none of this iron were stored but converted entirely into hemoglobin.

The polycythemic patient came into the ward during a remission stage. He had shown 10,000,000 red blood cells a few months previously and had been treated by radiation and phenylhydrazine with good response. When he entered the metabolism ward his red cell count was 5,300,000 and his hemoglobin was 80 per cent. Not until he had been studied for almost 4 months did his red cells begin to increase appreciably so that the effects of therapy on his iron metabolism

could be followed. Therapy consisted first of Fowler's solution according to the method of Forkner ('32). It was found that although his count could be brought down to 5,000,000 the effective dose was so close to the toxic one that medication had to be suspended. At the end of nine periods his red cell count was again 7,000,000 and his hemoglobin rose to 125 per cent.

The patient was now given intensive x-ray therapy, eleven treatments within 24 days. His red cell count dropped to 5,300,000; his hemoglobin to 87 per cent. But 1 week later his count rose to 6,100,000 and 113 per cent hemoglobin.

For two periods the patient was given no medication or treatment. He was now put on large doses of phenylhydrazine, 0.3 gm. every day for 7 days, a total of 2.1 gm. His red cell count dropped in about 2 weeks after cessation of the therapy to 2,900,000 and his hemoglobin to 56 per cent. When he was discharged 24 days after the medication was withdrawn his red cell count was 3,600,000 and his hemoglobin was 59 per cent.

During a preliminary study the subject excreted in his stool during one period as much as 167 mg. of iron daily. This was never found again during the observation and is mentioned here because it is possible that stored iron may be excreted in large amounts over short periods of time in polycythemic patients.

Table 2 and chart 2 summarize the findings obtained in this patient.

One of the striking features of the polycythemic patient's findings was a lack of correlation between iron intake and iron storage. On a higher intake he stored less iron than on a relatively lower intake at the start of the experiment. Of course we have no knowledge of the state of his storehouses before the experiment began except that in the preliminary tests he excreted relatively large amounts of iron. During his periods of therapy he was constantly on a slightly negative balance but even with a tremendous decrease of 50 per cent of his red blood cells and hemoglobin which occurred with

phenylhydrazine administration his output of iron was insignificant. This substantiates fully the findings of Bassett, Killip and McCann ('31). He actually lost during this period 79.2 mg. of iron. To account for his blood drop he should

TABLE 2

NUMBER OF PERIODS	AVERAGE DAILY IRON INTAKE (MG.)	AVERAGE DAILY TOTAL EXCRETION (MG.)	AVERAGE DAILY IRON BALANCE (MG.)	BLOOD COUNT		COMMENTS
				Hb. per cent (15.8 gm. = 100 per cent)	R.B.C. in millions	
5	12.8	9.6	+3.2	90	5.5	
				83	4.8	
				76	5.5	
3	14.1	15.7	—1.6	84	4.8	
				93	5.1	
6	12.1	8.9	+3.2	99	5.3	
				95	5.8	
				100	5.6	
12	18.1	16.7	+1.4	105	6.1	
				105	7.4	
				110	6.8	
10	17.6	17.9	—0.3	99	6.5	Fowler's solution daily for seven periods; very little in last two periods
				120	5.0	
				118	7.0	
6	18.6	22.1	—3.5	95	6.5	X-ray, 11 treatments in 24 days
				95	5.5	
2	19.1	20.5	—1.4	87	5.3	
				113	5.5	
8	15.9	19.2	—3.3	88	6.1	Phenylhydrazine, 0.3 gm. daily for 7 days in first two periods
				56	2.9	
				59	3.6	

have lost 2100 mg. of iron. This difference was undoubtedly stored.

One of the features of this experiment is the constancy of iron excretion per gram of dried stool. In the normal subject on an iron intake between 10 and 25 mg. the dried stool per

gram varied in iron content between 0.36 mg. and 0.82 mg. with an average of 0.6 mg. On an 80-mg. intake the variation was between 38.4 and 64.0, averaging 48.7 mg. In the case of the polycythemic patient all through the experiment except in the preliminary phase, the variation of iron per gram of dried stool was between 0.32 mg. and 0.78 mg., with an average of 0.51 mg.

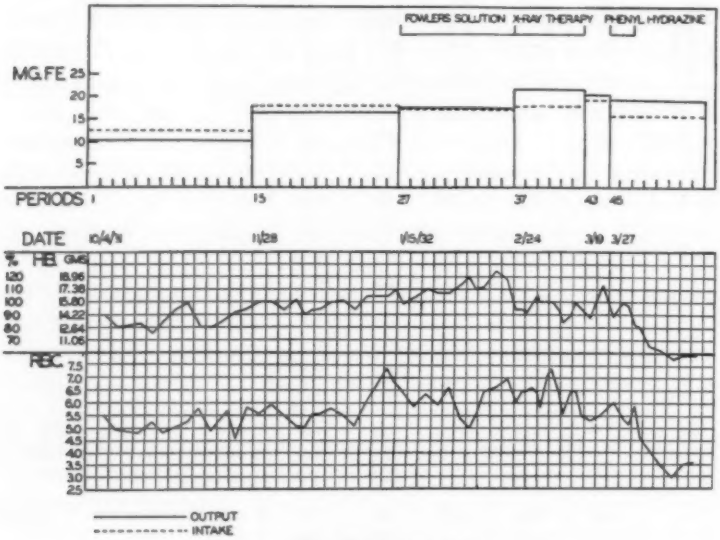

Chart 2. Polycythemic patient

The daily urine excretion of iron in the normal person varied from 0.2 mg. to 0.62 mg., averaging 0.35 mg. In the polycythemic patient daily iron urinary excretion varied from 0.24 mg. to 0.74 mg., averaging 0.42 mg.

CONCLUSIONS

1. A normal subject was found to store iron when placed on an intake of 26 mg. daily and was in balance when on a 10-mg. intake before any therapy was started.

2. A parenteral liver extract, which is potent in causing a remission in pernicious anemia, appeared to cause a retention of iron when the subject was on a 10-mg. intake and an increase in iron storage when he was on a 17-mg. intake. When he was put on a high iron intake, approximately 80 mg., administration of parenteral liver extract was followed by a marked increase of iron excretion. Copper sulfate seemed to decrease the iron loss.

3. Administration of a liver fraction, supposedly potent in 'secondary' anemia, was followed by a retention of iron over and above that obtained when iron in quantities present in the liver fraction alone was given.

4. Administration of massive doses of iron (2 gm.) gave a marked retention of iron.

5. During a remission stage a polycythemic patient was on a slightly positive iron balance when his intake varied from 14 to 18 mg.

6. Even with a decrease of 50 per cent of his blood following phenylhydrazine no marked increase of iron excretion was obtained. This suggests that the great bulk of iron so liberated is stored in the body.

7. Iron excretion in the urine is little and remarkably constant and cannot be increased by any method tried in these experiments.

8. The amount of iron excreted per gram of dried stool is remarkably constant except when large amounts of iron are given.

9. The red blood cell count and hemoglobin values had only an indirect relationship to iron intake and therapy in the normal or polycythemic subject because of the intermediation of the iron storage depots.

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HUMAN MILK STUDIES

XII. THE VITAMIN B AND VITAMIN G CONTENT BEFORE AND DURING MATERNAL CONSUMPTION OF YEAST [1]

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TWO FIGURES

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The vitamin B potency of either human or cow's milk alone may not always be sufficient for the consistent growth and well-being of the infant (Hoobler, '28; Rose, '30). Shortcomings are recognized by the appearance of symptoms in the child, varying from the loss of appetite and failure to gain in weight to the manifestation of a definite syndrome of beriberi, symbolizing the lack of the vitamin B complex. Moreover, clinical observations have shown that alleviation of these symptoms follows either the administration of this nutrient, extract of vegetable soup (Daniels, Byfield and Laughlin, '19), wheat germ sugar (Dennett, '29; Waring, '29, '31; Henricka, '32; West, '29; and Berney, '31), or, yeast (Waring, '29, '31; West, '29; Bloxsom, '29) to the infant directly or indirectly by fortification of the diet of the mother (Henricka, '32; West, '29; Brenneman, '23). Since this vitamin moiety can neither be synthesized nor stored in the

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[2] The data comprise the dissertation submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in the Division of the Biological Sciences, The University of Chicago.

body to any appreciable extent, the maternal diet then must serve not only as the sole supply for maintenance and increased physiological demands of lactation, but also for the vitamin content of the milk.

Although the optimal vitamin requirement of the infant is not known in specific quantitative units, the significant fact is that dietary insufficiencies actually occur in some infants fed either with breast or cow's milk. In many of the clinical studies on human milk the diets of the mothers were known to be notably inadequate. But even the milk from women consuming diets with average amounts of the vitamin B complex has been found by biological assay to contain a relatively low concentration of this substance (Macy et al., '27). Improvement may be effected also in cow's milk formulae fed routinely in the hospital by the addition of specific vitamin adjuvants, as evidenced by the stimulation of a better appetite and increased growth in the infant. These demonstrations that the infant may experience frank dietary deficiencies with milk as the chief source of food, and that there are, no doubt, borderline conditions with symptoms yet unclassified or even unrecognized make it evident that positive means should be devised for insuring the child against alimental inadequacies during the critical period of rapid growth, thereby safeguarding not only present, but future nutritional stability.

It has been demonstrated experimentally that the maternal requirement for vitamin B is augmented during lactation (Macy et al., '27; Evans and Burr, '28; Sure, '27) from three to five times over and above that during growth. In the human mother with a failing or small milk supply, the addition of yeast and yeast preparations to the diet has been observed to promote an increased flow of milk (Waring, '31; Henricka, '32; and Wachtel, '30). On the other hand, women who were successful in the production of large quantities of breast milk experienced no greater output with supplementation of their diet, but a quantitative assay of the biological potency of the milk disclosed an improvement in its vitamin content (McCosh, Macy and Hunscher, '31). At the same

time the mothers themselves expressed a feeling of lessened fatigue with a general stimulation of well-being (Macy et al., '30).

The augmentation of the diet of the mother with yeast or yeast preparations benefits both mother and child, and since it is recognized that there are at least two well-defined water-soluble vitamins in yeast, it is pertinent to question whether each bears equal influence in the improved condition or whether one portion is more helpful than the other. Laboratory experimentation has indicated that for successful lactation and rearing of young rats, increased amounts of both the antineuritic vitamin B and of vitamin G are needed (Sure, '28). Some investigators have found a greater need for the former (Evans and Burr, '28) and others for the latter (Husseman, '31), but no study as yet has been reported on women. There are clinical observations, however, indicating that the rapidly growing infant requires larger amounts of each of these vitamins than is furnished by milk, for the feeding of accessory food in addition to the milk resulted in some cases in a promotion of growth in the infant with no increase in total food consumption (Hess, '17), in others the appetite was stimulated as well as growth, while in others the symptoms of rigidity and beriberi were mitigated. The improvement in the infant accompanying the enrichment of the maternal dietary may be due to a selective augmentation of one or the other or both of these two well-recognized vitamin constituents or to some unidentified factor in breast milk. From experimental observations in this laboratory (McCosh, Macy, and Hunscher, '31) there are indications that the fractions may be increased disproportionally. When the average diets of lactating women were supplemented with yeast, some factor promoting better utilization of food was increased in the milk. The factual evidence was demonstrated by the behavior of experimental animals in that equal or better gains in body weight were achieved on fewer calories per square meter of surface area for 24 hours during the period of maternal yeast consumption.

Since the vitamin requirement for lactation has not been measured quantitatively for woman, and there is experimental evidence to indicate that the demand for the vitamin B complex is increased at this time, further enrichment of the average dietary in this essential food component may approach a more nearly optimal food provision for both the mother and her dependent infant. By a quantitative assay of each of the constituents of this vitamin moiety passed into milk, concomitant with the supplemented maternal dietary, the ingredient which is instrumental in improving the nutritive potency of the milk may be recognized. Data derived from such an assay may aid in assuring "a more nearly perfect food for the infant," one which will supply all of the nutritive needs of the young child, thus stimulating his optimal physical progress.

The present report is a study of the effectiveness of diets rich in the vitamin B complex on the nutrition of women during lactation and on the biological potency of the milk secreted coincidentally. It includes quantitative tests on the vitamin B and vitamin G[3] content of breast milk of an individual as well as of mixed milk from a group of women before and after their accustomed menus were supplemented with yeast. In addition, data are given on the relation of growth of experimental animals to the vitamin and total caloric ingesta, as well as on the composition of growth as measured by nitrogen balance studies.

EXPERIMENTAL

Subjects and breast milk. The women chosen to provide the milk for this investigation were able to secrete enough to feed their own infants and to supply for the Mother's Milk Bureau of Detroit approximately 13 ounces in excess daily. Observa-

[3] The terms for the different vitamins as used in this report are defined below for clarity in discussion; vitamin B complex is the undifferentiated vitamin originally designated as vitamin B; vitamin B is the antineuritic substance for which the British use the term vitamin B_1; vitamin G is the more heat-stable substance or substances known originally as the 'Goldberger Factor' and which is specified as vitamin B_2 by the British.

tions were begun in the second or third month of lactation and extended over a period of 6 to 8 months thereafter, during which time they maintained their usual output of milk. The maternal food and liquid intakes were measured 1 week out of each month. All of the diets during the period of observation were found to be adequate according to present accepted qualitative dietary standards (Sherman, '32). The milk secreted during the period in which the women were eating their accustomed diets and that produced after 6 weeks of consumption of supplements of 10 gm. of yeast daily were tested for vitamin B and vitamin G potency.[4] At 9 o'clock each morning the milk was manually expressed at the Bureau, and the containers sealed air tight and packed in ice until the milk was fed at 11 A.M. The yeast supplement was administered at the Mother's Milk Bureau. The infants of the women were healthy throughout the observation and made for the most part average gains of about 6 ounces per week.

For statistical interpretation and the assurance of representative growth, large numbers of test animals are required for biological assay work, consequently a greater amount of test material must be available. The quantity factor becomes more significant in testing mother's milk for vitamins B and G, since not only must large numbers of animals be maintained, but considerable amounts of milk are required for the several graded levels fed. Human mothers, as a rule, do not produce much milk in excess of that needed by their own babies, hence, in order to secure a sufficiency for reliable biological tests, pooled milk from a group of women must be used, although certain inherent complications are introduced. In view of the fact that there seems to be a direct relationship between the concentration of the vitamin B complex and the quantity of milk secreted (McCosh, Macy, and Hunscher, '31; Macy, Outhouse, and Hunscher, '28), it is of

[4] For convenience the period during which the mothers were on their usual diets will be designated as the pre-yeast period and that following and during the addition of yeast to the maternal diet as the yeast period. The vitamin G potency of 10 gm. of yeast is equivalent to 75 to 150 units, according to Sherman.

importance in a study of potency of the vitamins B and G in mixed milk, and in particular the effectiveness of dietary supplements upon the biological properties, to secure women of comparable capacities for milk production. In the mixed sample, a change in the vitamin concentration of one individual milk may be concealed by the diluting effect of the lack of change in another. This indicates the advisability of studying individual milks as a control even though it becomes necessary to use fewer samples because of the diminished quantity of available sample. In this study not only mixed milk combined under standard conditions but also the milk of an individual mother as a control was used.

Experimental animal technic. Young albino rats reared on a stock diet of whole wheat and whole milk powder (Sherman and Campbell, '24) were placed in individual cages at approximately 28 days of age and given weighed portions of the experimental basal diet which they were allowed to consume ad libitum, except in one series of studies in which the quantity was controlled. The basal diet (Chase and Sherman, '31) fed for determining the vitamin B content of milk contained casein 18, dextrin 53, autoclaved yeast 15, filtered butterfat 8, cod liver oil 2,[5] Osborne and Mendel salt mixture 4 (Osborne and Mendel, '19); and for determining vitamin G (Bourquin and Sherman, '31) casein 18, butterfat 8, cod liver oil 2, Osborne and Mendel salt mixture 4, and dextrin 68, on which was dried an alcoholic extract of whole wheat in such proportion as to introduce the extract (80 per cent alcohol by weight) of 50 gm. of whole wheat into 100 gm. of food mixture. For the most part from six to ten standardized animals were maintained on each test level of milk over an 8-week experimental period following a period on the vitamin-free diet during which the exhaustion of the store of vitamins B and G in the animal body took place.

Only those animals that consumed all of the milk daily are included in the final averages of the weekly weight increments. It happens that the milk of some women is eaten with greater

[5] The kindness of Arthur D. Holmes, Ph.D., of the E. L. Patch Company, Boston, in supplying the cod liver oil is gratefully acknowledged.

avidity than that of others and this distaste of some animals for the milk persists throughout the test period, thus precluding the use of these results in the final interpretation of the data. With the larger quantities of breast milk as the source of the antineuritic vitamin, diarrhea frequently occurs in contrast to almost complete freedom from this condition with analogous quantities of milk supplying vitamin G. This fact is significant and is in accord with the findings that debilitation and lessened muscular tone of the intestinal tract accompany sub-optimal intakes of the antineuritic vitamin

TABLE 1

LEVEL OF MILK	PRE-YEAST PERIOD				YEAST PERIOD			
	Number of animals	Mean weekly gain	Mean weekly basal food intake	Mean weekly gain 100 cal. food	Number of animals	Mean weekly gain	Mean weekly basal food intake	Mean weekly gain 100 cal. food
Vitamin B-free diet + mixed breast milk								
Cc.		*Gm.*	*Gm.*	*Gm.*		*Gm.*		
10	10	2.5	28.8	1.8	6	2.7	31.5	1.6
20	10	8.5	31.4	3.4	6	7.4	36.0	3.1
25	5	8.9	28.7	3.4	7	8.7	26.3	3.6
Vitamin G-free diet + mixed breast milk								
3	10	2.0	27.3	1.4	8	2.9	30.5	1.9
5	10	2.5	26.8	1.8	8	6.0	36.0	3.2
10	10	7.8	35.3	3.6	7	7.3	35.6	3.4
20	12	11.5	44.3	3.7	7	9.6	30.3	3.9

(Rose, Stucky and Cowgill, '30; McCarrison, '21). The high concentration of lactose in mother's milk may also be a mediating factor (Macy et al., '27). Because of these vitiating conditions, tests on the antineuritic vitamin potency of human milk are less conclusive than those on the vitamin G content.

Basal diet ad libitum. Equal quantities of breast milk from women with similar production records were combined daily to form the sample of mixed milk. Individual standardized test animals were given varying quantities of milk from 10 to 25 cc. daily as the sole supply of the antineuritic vitamin in a diet otherwise complete, and 3 to 20 cc. for the vitamin G test (table 1). The smaller quantities allow for the deter-

mination of the unit growth suggested by Sherman (Chase and Sherman, '31; Bourquin and Sherman, '31) and the increased amounts permit a study of the influence of more adequate vitamin intakes.

In both the vitamin B and vitamin G studies there was a progressive increase in the growth of the test animals coincident with increased consumption of milk as a source of these vitamins. This was true whether the milk was secreted by the mothers in the pre-yeast period or in the yeast period when supplements of 10 gm. of yeast were being consumed daily along with their ordinary diets.

With 3, 5 and 10 cc. of mixed milk daily as the source of vitamin G, the growth of the animals in the yeast period was equal to, or superior to, that of the pre-yeast period. The mean weekly gain of the standardized test animals over an 8-week period for the daily intakes of 3, 5, 10, and 20 cc. of mixed breast milk was 2.0 2.5, 7.8, and 11.5 gm., respectively, for the animals in the pre-yeast period and 2.9, 6.0, 7.3, and 9.6 gm., respectively, for the yeast period. The increased gain of the animals when they were receiving milk at the lower levels from the mothers consuming a dietary supplement of yeast demonstrates that the vitamin G is carried over into the milk in appreciable quantities. These findings are further borne out by a study of the milk from an individual woman in which case the mean weekly gain in weight of animals receiving 3, 5, and 10 cc. of milk daily was 0.3, 3.4, and 7.0 gm. for the pre-yeast period and 3.6, 4.2, 7.9 gm., respectively, for the yeast period. Divergent results were at times experienced when quantities of 20 cc. of milk daily were fed for vitamin G. These inconsistencies with the higher level of milk may possibly be ascribed to the exhaustion of the store in the animal body of some yet unidentified food factors necessary for the growth of the rat as suggested by Bourquin and Sherman ('31) and hence these results may not be as reliable as those for the lesser quantities fed.

In contrast to the experiments on the vitamin G content of breast milk, the ingestion of yeast by the mothers seems to

exert no perceptible influence on the concentration of vitamin B in the milk secreted. Animals getting quantities of breast milk as high as 10 cc. daily developed polyneuritis in approximately one-quarter of the cases both before and after supplemental additions of yeast to the maternal dietary. On higher levels of milk, 20 and 25 cc. daily, results were complicated by failure to take all of the milk and also by the occurrence of diarrhea. The mean weekly gains of those animals consuming the full quota of milk daily, irrespective of other conditions, were for the animals in the pre-yeast period getting 10, 20, and 25 cc. of mixed milk, respectively, 2.5, 8.5, and 8.9 gm., and for the comparable animals in the yeast period, 2.7, 7.4, and 8.7 gm. After eliminating from the final average the gains of all animals ill-conditioned either by the failure to consume all of the milk daily or by the diarrheal condition, the resultant mean weekly gains for the remaining animals in the pre-yeast group getting 10, 20, and 25 cc. of milk daily were, respectively, 2.5, 8.8, and 10.4 gm., and for the animals in the yeast period 2.7, 9.0, and 10.6 gm. As judged by the rate of growth of rats, no discernible increase in the antineuritic potency of the breast milk herein studied was effected by the addition of 10 gm. of yeast to the maternal dietary of average vitamin content.

The weekly gain of the test animals was studied in relation to their caloric food consumption in order to further verify the growth rates of the animals in the pre-yeast vs. the yeast period, and to ascertain whether these increments were the result of a difference in the utilization of food or of an increased food intake. As based upon gain per 100 calories of food consumed, the test animals getting vitamin G from mixed milk at the lower levels manifested a noteworthy increase during the yeast period, whereas, no improvement could be recognized in those animals having milk as a source of vitamin B (table 1). These findings are confirmatory of the results of a previous study in this laboratory (McCosh, Macy, and Hunscher, '31) in which a more efficient use of food was similarly observed in experimental animals receiving breast

milk secreted during the addition of yeast to the diet of lactating women. The present study demonstrates that this improvement was due to the augmentation of the vitamin G fraction.

With enrichment of the maternal diet with 10 gm. of yeast daily, there occurred an increase in the vitamin G content of the breast milk, but there was no detectable improvement in the vitamin B potency. The vitamin G content was approximately 0.2 unit per cubic centimeter and the vitamin B concentration 0.1 unit per cubic centimeter before the supplementation of the mother's diet, but after the maternal consumption of yeast the concentration of vitamin G in the milk was increased to 0.3 unit per cubic centimeter. The specific causal factors for the disproportionate improvement in these biological components of the milk is enigmatical, and deserves detailed investigation. From their work on cows and goats, Gunderson and Steenbock ('32) concluded that the maximum concentration of vitamin B in milk is under physiological control, for when the content of this vitamin in the ration was increased from a reasonably good level to approximately 66 per cent more by the addition of yeast and wheat germ, the concentration of the antineuritic factor in the milk was unchanged. A similar interpretation may be applicable to the observed constancy of the vitamin B potency of human milk.

Controlled intake of basal diet. From the growth studies in which the basal food was consumed ad libitum, it was apparent that gain in weight of the experimental animals, although primarily influenced by the quantity of vitamin fed, varied somewhat with the amount of basal food consumed (table 1). Variability in the quantity of basal food intake is inherent in an experiment in which the food is fed ad libitum and which factor Irwin, Nelson et al. ('30) have found to bear a significant influence on total gain in body weight. For this reason a series of observations with two groups of rats of ten each were conducted on the vitamins B and G content of the milk of an individual mother fed at 5 and 15 cc. levels daily before

and during the addition of yeast to her diet in which the total caloric allowance of food for the test animals during the yeast period was maintained at the same level per gram of body weight as during the pre-yeast period. The observation extended over 7 weeks, divided into a 2-week pre-yeast period followed by a 5-week yeast period. During the last 3 weeks of the latter the amount of milk supplied daily to each group of rats was increased to approximate the mean amount fed per gram of body weight during the pre-yeast period, because of the recognition that some relationship exists between body weight and the vitamin B requirement (Osborne and Mendel, '22; Cowgill et al., '32). Under these controlled conditions there was a marked acceleration in the growth of animals having vitamin G supplied by breast milk during the ingestion of yeast by the mother; whereas, the rate of gain in body weight of the animals with milk as a source of vitamin B was not significantly changed (chart 1). The yeast supplementation had been made to the mother's diet only 2 weeks prior to the experimental observations—a shorter length of time than in the other studies of this report—but even in this case there was an increase in the vitamin G potency of the milk as judged from the marked increase in gain in body weight of the test animals.

Human milk, as determined by quantitative biological tests, is a comparatively richer source of vitamin G than of vitamin B. With the same quantity of breast milk supplying each of these factors in the respective vitamin-free diets, the growth of the animals with vitamin G supplied by milk was superior to that of those with milk as the source of vitamin B (chart 2). By relating the increment in weight of each group of test rats to their food intake (gain per 100 calories of food consumed) the growth of those animals having vitamin G from breast milk surpassed that of those receiving the antineuritic vitamin from the same amount of milk irrespective of the maternal dietary (table 1). The mean weekly gain in body weight per 100 calories of food intake of the animals with 10 cc. of breast milk daily as a source of vitamin B was 1.8 gm. in contrast

to a gain of 3.6 gm. in those rats with milk as a source of vitamin G; the gains during the yeast period were 1.6 and 3.4 gm., respectively. With 20 cc. of breast milk daily supply-

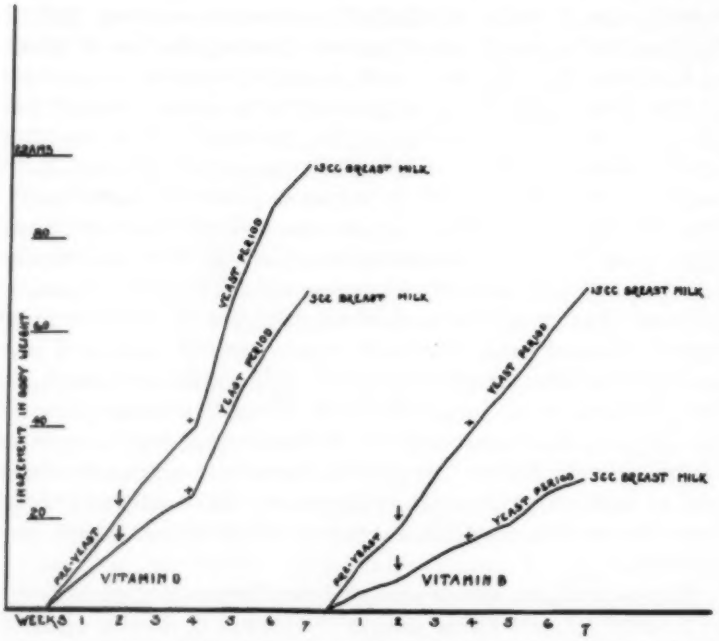

Chart 1 This chart illustrates the average gain in weight of groups of test rats maintained on an approximately constant caloric food intake per gram of body weight with 5 and 15 cc. of milk daily per rat from an individual woman as the source of vitamins B and G in a diet otherwise complete during the pre-yeast period and the equivalent amounts in terms of grams of body weight to compensate for the increased size of the animal during the yeast period. The arrow indicates the introduction of 10 gm. of yeast daily into the maternal diet.

ing vitamins B and G, the mean weekly gains of experimental animals per 100 calories of food consumed were 3.4 and 3.7 gm., respectively, during the pre-yeast period and 3.1 and 3.9 gm., respectively, during the yeast period.

A quantitative comparison of the vitamin B and G content of breast milk is possible by ascertaining the amounts of milk required as a source of each of these vitamins to support unit growth (an average gain of 3 gm. per week for 8 weeks). As a source of vitamin B, 10 cc. of milk gives an average gain in weight per the unit rate in the experimental animals during

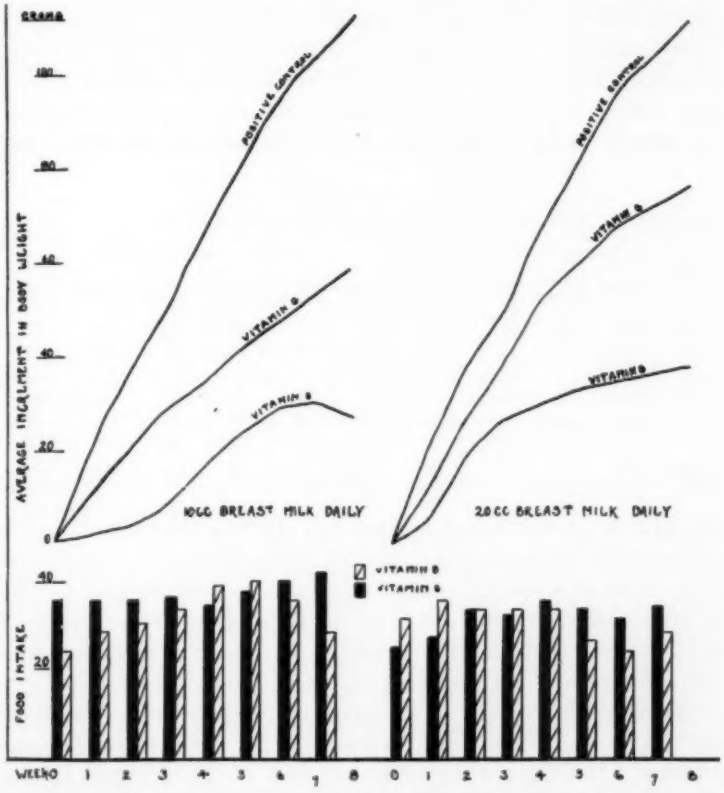

Chart 2 This chart compares the vitamin B and G potency of breast milk. In neither case, however, did the animals receiving as much as 20 cc. of milk daily per rat grow as satisfactorily as the positive controls. The animals consuming milk as the source of vitamin B ate somewhat less basal food than those in the vitamin G test.

both the pre-yeast and the yeast period, despite the occurrence of polyneuritis in approximately one-quarter of the cases, whereas, only 5 cc. daily are required for unit growth in the vitamin G test animals before supplementation of the maternal diet, and only 3 cc. after the introduction of yeast into the diets of the mothers.

Composition of growth of animals. Since the composition of growth is known to be variable, it seemed pertinent to investigate the type of gain in weight made by test animals receiving vitamins B and G from breast milk secreted before and during enrichment of the maternal diet in these factors. By means of 7-day nitrogen metabolic balance observations,

TABLE 2

Retention of nitrogen in per cent of intake

WEEK	VITAMIN B-FREE DIET + MILK				VITAMIN G-FREE DIET + MILK					
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	21	39	37	43	42	65	29	61	42	57
2	31	40	24	44	54	57	30	58	54	50
3	Loss	46	No balance	47	43	49	35	55	43	54
4	Loss	38	9	33	38	42	33	48	38	44
5	14	46	11	34	37	41	35	53	37	43
Mean	22	42	20	40	43	51	32	55	43	50

the milk of an individual woman was studied over 5 continuous weeks while the mother was on her usual dietary, and followed by a study of a similar period when she was consuming 10 gm. of yeast daily. Each animal of the latter period was matched as to weight with an animal of the pre-yeast period and was fed both basal food and vitamin in the same proportion per gram of body weight. Two pairs of animals were observed for the vitamin B test and three pairs for the vitamin G comparison. In all cases a greater per cent of the nitrogen intake was retained by the animals receiving milk from the mother during her period of yeast consumption (table 2). Conservation of the ingested nitrogen was accomplished by a lessened urinary nitrogen excretion (table 3) with no consistent

changes occurring in the fecal nitrogen as indicated by the coefficient of digestibility. The mean nitrogen retentions for each gram of gain in body weight for the paired animals in the pre-yeast period and those in the yeast period getting vitamin B from milk were 20.0 and 28.0 mg., 30.0 and 35.0 mg., respectively, and for those fed milk as a source of vitamin G, 34.0 and 38.0 mg., 31.0 and 39.0 mg., and 34.0 and 39.0 mg., respectively. The composition of the growth of the test animals receiving breast milk as the source of vitamin G was of higher nitrogen content during both the pre-yeast and the

TABLE 3

Excretion of nitrogen in urine in per cent of intake

WEEK	VITAMIN B-FREE DIET + MILK				VITAMIN G-FREE DIET + MILK					
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	64	48	46	39	49	25	60	30	49	31
2	52	51	58	35	35	34	56	29	35	38
3	78	45	63	34	47	39	51	34	47	36
4	82	48	70	45	49	44	53	40	49	44
5	60	55	66	49	50	45	51	36	50	46
Mean	67	49	61	40	46	37	54	34	46	39
Coefficient of digestibility	77	87	81	80	89	88	87	89	89	89

yeast period than that of the animals consuming a like quantity of milk for vitamin B.

Since it has been demonstrated that enrichment of the maternal dietary confers added nutritive properties to the milk, it is of interest to know the simultaneous effect that occurs in the metabolism of the mother. Nitrogen balances of lactating women determined in this laboratory before and during the supplementation of their usual diets with yeast indicated that enhancement in the biological properties of the milk did take place (McCosh, Macy, and Hunscher, '31) and in some cases was accompanied by improved storage of nitrogen by the mother herself (Hunscher et al., '33).

SUMMARY

1. Individual and mixed samples of breast milk from women with approximately the same milk flow and on apparently satisfactory dietaries were tested biologically by means of several different controlled methods for their vitamin B and vitamin G potency before and during the supplementary additions of 10 gm. of yeast daily to the accustomed maternal dietary.

2. In both the vitamin B and vitamin G studies there was a progressive increase in the growth of the test animals simultaneously with increased consumption of breast milk. This was true whether the milk was secreted during the pre-yeast period or the yeast period.

3. The concentration of vitamin G in breast milk was increased from 0.2 unit per cubic centimeter during the pre-yeast period to 0.3 unit during the yeast period; an increase of approximately 0.1 unit per cubic centimeter, by the addition of 10 gm. of yeast to the maternal diet for the milk tested.

4. As judged by the rat growth rate method, no increase in the antineuritic potency of breast milk occurred under yeast therapy. The concentration of approximately 0.1 unit of vitamin B per cubic centimeter of breast milk remained unchanged.

5. By a series of observations in which the caloric food consumption of the experimental animals was controlled, further evidence was given that the vitamin G concentration is increased, but that no change occurs in the vitamin B content of breast milk with this enrichment of the maternal diet.

6. Nitrogen metabolic balances on test rats receiving vitamins B and G from breast milk, before and after the addition of yeast to the maternal diet, showed a greater retention of nitrogen during the latter period in all animals. The composition of the growth of the animals with vitamin G from breast milk was higher in nitrogen content than the growth of the animals with vitamin B supplied by equal quantities of milk.

7. From the observations on the women of this investigation, it can be concluded that the biological potency of breast milk can be enhanced by the addition of a concentrated source of the vitamin B complex to the average dietary. At the same time, the mothers themselves experienced less fatigue and a more satisfactory feeling of well being. These studies further suggest the need for more adequate knowledge of diet for lactating women in order that an approach can be made toward a nearly optimal food provision for both the mother and her dependent infant.

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